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ABSTRACTS

O-1

HODGKIN'S DISEASE IN CHILDEN - THE ROLE OF EBV AND HIV IN PATHOGENESIS

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Despite significant advances in treatment, the aetiology of Hodgkin's disease (HD) has remained elusive. The Epstein-Barr virus (EBV) may play a role in the aetiology of HD. A prime candidate for the cell of origin could be the B lymphocyte (CD 20, CD 79a) and interdigitating reticulum cell (IRC). Hodgkin and Reed-Sternberg (HRS) cells found in patients with HD may represent in vivo hybridomas of the IRC with B- and/or T-cells. As IRC is unlikely to be susceptible to EBV, a retrovirus may be the culprit. Hybridomas may be formed when retroviral antigens expressed by a macrophage cell attract reactive B- and T-cells and, instead of an immune reaction, fusion occurs. Expression of the Epstein-Barr virus (EBV) gene product latent membrane protein 1 (LMP1) has been found in Hodgkin and Reed-Sternberg cells (HRS) in varying proportions of HD cases. It has been shown that LMP1 has transforming effect and could be responsible for creating HRS cells. To determine the role of EBV in childhood HD in different geographical areas, we used immunohistochemical staining to analyse LMP 1 in childhood HD from 10 different countries, in the addition 19 formalin fixed, paraffin embedded lymph nodes from Kenyan cases were screened for HIV using PCR. The proportion of LMP 1 positive cases varied significantly being 50% of cases from the U.K. (38/75), South Africa (9/18), Egypt (7/14) and Jordan (8/16), 60% from the United Arab Emirates (6/10), 70% from Australia (11/16), 81% from Costa Rica (34/42), 88% from Iran (7/8), 90% from Greece (20/22), and 100% of the 56 cases from Kenya. However we found so far only one HIV positive case of 19 Kenyan HD using PCR.

Our results support the hypothesis that HD in children and young adults has different aetiologies and that EBV is more likely to be involved in the pathogenesis of paediatric cases particularly in countries with lower socio-economic status.

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O-2

DISTRIBUTION OF t(8;14)(q24;q32) BREAKPOINTS IN EUROPEAN CHILDHOOD BURKITT'S LYMPHOMA AND B-ALL: A PCR BASED ANALYSIS

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Burkitt's Lymphoma and B-ALL are characterized by a reciprocal chromosomal translocation involving the protooncogene c-myc on chromosome 8q24 or one of the immunoglobulin constant region genes, either on chromosome 14q32, 2p12 or chromosome 22q11. In both endemic BL (eBL) of African origin or sporadic BL (sBL) from Northamerica and Europe, the translocation t(8;14)(q24;q32) is the most common form.

For molecular characterization of the breakpoints, we analyzed 49 childhood Burkitt's lymphoma, collected during trials NHL-BFM '90 and '95, using a long distance PCR-approach. In 36 of 49 samples products between 800 and 8500 bp in length could be amplified using primers from the JH, Sμ, Cμ, Cε, Cγ, and Cα- regions together with a c-myc specific primer. The following distribution of breakpoints on chromosome 14q32 were observed:

chromosome 14	JH	Cμ/Sμ	Cγ/Sγ	Cα/Sα	Cε
patients	8	6	7	15	0

All breakpoints on chromosome 8q24 were localized within or 5' of the noncoding region of the c-myc gene. In our population of European sporadic BL we found a strong association of breakpoints within the Cα/Sα-region consistent with the report of Saglio et al (Genes, Chromosomes & Cancer, 8, 1993). In addition, a high incidence of breakpoints was seen in the JH-region, previously reported to be a feature of endemic BL.

Our data suggest that European cases of sBL differ in their distribution

of IgH-breakpoints from Northamerican cases of sBL, as reported by Shiramizu et al (Blood, 77, 1991). This indicates distinct geographic subgroups of Burkitt's lymphoma within the sporadic form of the disease.

O-3

RESULTS OF CYTOGENETIC AND FLOWCYTOMETRIC INVESTIGATIONS OF LYMPHNODE BIOPSIES IN CHILDHOOD LYMPHOMAS

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During the period January 1 1992 to October 31 1996, 26 consecutive cases of malignant lymphoma (Hodgkin lymphoma (HD): n=12 and non-HD: n=14) were diagnosed at the Institute of Pathology, Odense. The cases are thought to represent all or almost all cases from an area covering around 1/5 of the Danish population diagnosed in the mentioned time period. Patients were aged between 4½ years and 18 at the time of diagnosis.
Cytogenetic investigation was carried out in 14 and concomitant flowcytometry in 7.
Results: Metaphases for detailed karyotyping and/or determination of modal number was obtained in all cases (successrate: 100%). 12 (85%) showed clonal abnormalities: 3/3 HD, 5/5 B-lymphomas and 3/3 anaplastic large cell (ALC) lymphomas. Only 1/3 T-lymphoblastic (T-lb) lymphomas revealed an abnormal clone.
Patients with HD showed complex karyotypes with aberrations of chromosome 1, 6, 7, 16 and 19. Patients with B-lymphomas (Burkitt: n=2, centroblastic: n=1, immunoblastic: n=1, B-lymphoblastic: n=1) showed t(8;14) and t(8;22) in 2 and 2 cases, respectively. The B-lymphoblastic lymphoma showed 55-57inc, characteristic of B-ALL/B-lb. Patients with ALC all showed t(2;5), in 1 case as the sole abnormality (T-ALC).
Few metaphases with clonal abnormalities was characteristic of HD (2/25, 4/25, 10/25) as opposed to ALC (18/20, 27/27, 24/25). B-lymphomas showed varying nos. of abnormal metaphases.
Flowcytometric analysis was informative in 5/7 analysed cases. The 2 non-informative cases had abnormal cytogenetics. In these 2 cases (1 HD and 1 Burkitt lymphoma with partial lymphnode infiltration) morphology and immunohistochemistry was non-diagnostic, too (14%).
Conclusions: Cytogenetic investigation of malignant lymphoma is mandatory and seems superior to both morphology and flowcytometry especially in cases with partial lymph node involvement (2 of our cases). When a high success rate (100% at our institution) can be achieved it is possible to reveal a neoplastic clone in all high grade non-T lymphomas and in HD.
Cytogenetics may further be a useful tool for the subclassification of lymphomas which may have implications for treatment.

O-4

MOLECULAR MARKERS PREDICTING POOR OUTCOME IN MEDULLOBLASTOMA AND SUPRATENTORIAL PNET

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So far there are only few studies reporting diagnostic or prognostic relevance of chromosomal aberrations in childhood medulloblastoma and supratentorial primitive neuroectodermal tumors (PNET). Using micro-satellite analysis, Southern blot hybridisation and fluorescence in situ hybridization (FISH) techniques 28 primary tumors and 10 cerebrospinal fluid (CSF) metastasis probes were analyzed for loss of heterozygosity (LOH) of chromosomes 1q31, 6, 9q22, 10q, 11, 16q22 and 17p and for amplification of the c-myc proto-oncogene (c-myc^{amp}). LOH of 17p or hyperdiploidy of chromosome 17 was the most common aberration follwed by LOH 16q and c-myc^{amp}. Several tumors displayed aberrations of more than one locus.
Analysis of the clinical data showed that not one of the tumors with c-myc^{amp} (n=6) responded to chemotherapy, 4 of these patients have died. LOH of 17p or hyperdiploidy of chromosome 17 (n=22) was associated with one or several clinical risk factors (primary craniospinal metastasis, residual tumor after neurosurgery, poor response to chemotherapy). Two of three patients with LOH 9q have died. In contrast all patients with tumors without LOH 17p, LOH9q22 or c-myc^{amp} (N=9) are in first remission (mean observation time 22 months).
These results show, that molecular analysis of PNET may help to define high risk tumors. Prospective studies are necessary to determine if these analysis are useful markers for stratification of PNET to a less intensive or more aggressive therapeutic regimen.

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O-5

EFFECTIVE HUMAN BCL-2 ANTISENSE THERAPY FOR LYMPHOMA

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Many lymphomas have an inherent resistance to curative treatment mediated by altered patterns of gene expression from the apoptosis pathways. Difference within the lymphoma cell may be used to eradicate the malignant process. One approach, based on the "silencing" the BCL-2 gene (which prevention apoptosis), is antisense oligonucleotide therapy. Targeting the initiating codon of the BCL-2 gene, an 18 base antisense oligonucleotide (AO) sequence with a thioate chemistry to protect against nuclease activity was selected (G3139, developed with Genta USA). A period of 48 to 72 hours was required to doiwnregulate Bcl-2 protein. This was observed in lymphoma cell lines that overexpressed BCL-2 due to the t(14;18) translocation. Sense and nonsense oligonucleotides targeted to the same region showed none of these properties. Cellular internalisation to the nucleus and cytoplasm was good in a range of cells. *In vivo* pharmacokinetics and toxicity's were studied in rodents and primates. Toxicity's were minimal and tissue availability was maximal with continuous subcutaneous infusions, reaching a steady state at 72 hours with a 1μM plasma concentration at a dose of 5mg/kg/day. Good tolerance at a dose of 10mg/kg/day was seen in primates. Translation into *in vivo* lymphoma models using NOD/SCID mice to showed specific anti lymphoma effect at dose range from 1 to 10 mg/kg/day when infused over a two week period. A human phase I antisense study (using G3139) was commenced 18 months ago for patients with refractory B cell lymphoma characterised by high BCL-2 expression. Administration is by the subcutaneous route as a continuous infusion over a two week period (mimicking the *in vivo* model). The G3139 is well tolerated with minimal toxicity at doses up to a total dose of 6mg/kg/day. Toxicity's have been minimal and appear to be related to the thioate chemistry of the AO and not to antisense effects. The study is on going and as yet has not reached a maximum tolerated dose. Of the first 9 patients entered in the study, anti tumour activity has been observed 4, including one complete remission. The results suggest that down regulating the BCL-2 protein may provide therapeutic benefit where conventional chemotherapy has failed. Other strategies and improved AO targeted to BCL-2 may further advance this gene silencing approach in malignancy. The BCL-2 AO may eventually be used in combination with chemotherapy to overcome the chemoprotective effect of high BCL-2 expression. Gene silencing by antisense oligonucleotides has a role to play in lymphoma therapy.

O-6

CO-EXPRESSION OF THE DRT RECEPTOR KINASE AND ITS LIGANDS, LERKS, IN NEUROBLASTOMA.

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Receptor protein-tyrosine kinases (PTKs) of the EPH family are expressed highly and preferentially in the developing nervous system.

Evidence suggests that EPH-related PTKs and their ligands, LERKs, are involved in axonogenesis, axon guidance, acquisition of brain subregional identities, and neuronal cell survival. Some members of the EPH family PTKs have also been implicated in oncogenesis. We previously isolated and characterized a human *EPH*-related PTK gene, *DRT*. *DRT* maps to chromosome 1p36.1, a site of frequent deletion and rearrangement in neuroblastoma (NB). *DRT* transcripts are expressed highly in NB cell lines, compared to those of two other *EPH*-related genes, *ECK* and *NET*. Preliminary data have indicated that primary NB tumors express *DRT* mRNA as well. Interestingly, ECK has been shown to be frequently co-expressed with an ECK ligand, LERK-1, in metastatic melanoma cells, suggesting that the auto-activation of ECK in melanoma cells contributes to their progression.

Methods: To investigate if a similar co-expression situation exists with DRT and its ligands in NB, we performed Northern blot analysis on total RNA samples obtained from NB cell lines, using cDNA clones of DRT ligands (*LERK-2*, *LERK-5*, and *LERK-8*) as probes. These cDNA clones were isolated from a human fetal brain library using the mouse homologue of *LERK-2* as a probe.

Results: *LERK-5* and *LERK-8* transcripts were expressed in the majority of NB cell lines. Little expression of *LERK-2* mRNA was found in the NB cell lines tested.

Conclusions: These data suggest that auto-activation of the DRT receptor kinase occurs in NB cells that co-express DRT and its ligands. The auto-activation of DRT by its ligands may contribute to the pathogenesis of NB by providing NB cells with an increase in survival and/or growth advantage.

O-7

EXPRESSION OF MYELOID ANTIGENS IN CHILDHOOD ACUTE LYMOPHOBLASTIC LEUKEMIA (ALL) HAS NO PROGNOSTIC IMPACT IN BFM-ORIENTED AIEOP ALL 88 & 91 STUDIES

Putti MC, Basso G, Cocito MG, Biondi A, Conter V, Aricò M, Testi AM, Santoro N, Barisone E, Rondelli R, Rizzari R, Pession A, Zanesco L, Masera G for the AIEOP.

AIM: to determine the incidence of 6 myeloid antigens (MyAg) expression and to analyze its prognostic impact in children with ALL, treated at AIEOP institutions over 6 years.

METHODS: marrow cells of 908 patients with ALL were centrally phenotyped using lymphoid and 6 myeloid markers (CD11b, CD13, CD14, CD15, CD33, CD65w). Cases expressing at least one MyAg (>20% cells) were called MyAg+ALL; among them, cases positive for ≥2 MyAg (CD13, CD33, CDw65) were called Hybrid (HL). Univariate and multivariate statistical analysis was performed. 6-years EFS (median follow-up 42 months, range 19-87) was estimated.

RESULTS: 23 patients were infants, 726 were aged 1-9 years, 159 were 10-14. EFS was negatively correlated with age, high WBC count and poor response to prednisone ($p<0.0001$), but not with organomegaly, CNS involvement, hemoglobin level, platelets count or FAB morphology. Overall, 291/908 cases were MyAg+ALL (32%); 94 of the MyAg+ALL cases were HL. Their incidence was similar in B-ALL and T-ALL, and among Common, pre-B and pre-pre-B-ALL. CD13 and CD33 were most commonly found. Patients with MyAg presented standard risk features more frequently than other children; no difference was observed in prednisone response and CR rate. The 6 years EFS was 63.7% in the 291 patients with MyAg+ALL; 78.6% in the subgroup of HL and 69.0% in 617 patients with MyAg-ALL, without significant difference.

CONCLUSIONS: in this large series of centrally diagnosed ALL, homogeneously treated with BFM-oriented therapy, coexpression of myeloid antigens was not associated with prognostic significance.

O-8

GENE AMPLIFICATION IN HUMAN NEUROBLASTOMA - A REVERSIBLE PHENOMENON?

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Human neuroblastoma (NB) frequently exhibit N-*myc* amplification (NMA) and many of the cell lines are characterised by the presence of morphologically distinct cell types. The neuronal cells (N-cells) and the so-called flat cells (F-cells) are thought to represent manifestations of different neural crest cell lineages and are considered to be the consequence of NB cell pluripotency. In F-cells of NB cell lines with extrachromosomally amplified N-*myc*, we observed the frequent occurrence of micronuclei. Using fluorescence in situ hybridization (FISH) with an N-*myc* specific probe in a series of NB cell lines established in the CCRI, we demonstrate that these micronuclei were packed with N-*myc* hybridisation signals. In addition, in a minor percentage of cells, N-*myc* signals occurred in clusters, adhered to the nuclear membrane and aggregated in nuclear protrusions. In F-cells, a substantial reduction or lack of amplified N-*myc* copies was observed. These observations let us conclude that extrachromosomally amplified genes can be actively eliminated from the nucleus resulting in a dramatic loss of amplified sequences in the F-cells. Moreover, reduction or loss of amplified sequences in F-cells is shown to be accompanied by downregulation of N-*myc* expression, by a decrease in proliferative activity and by upregulation of molecules of the major histocompatibility complex class I (MHC I). In addition, the formation of F-cells can be increased by certain agents, e.g. BrdU, and furthermore, we have evidence that the mechanism of elimination of amplified genes can also operate *in vivo*. Interestingly, F-cells are not restricted to NB cell cultures but also occur in cell lines of other tissue origin. All F-cells share important biologic features, interpreted as cell revertance, i.e. loss of the malignant phenotype. This fact, together with the demonstration that NB cells do not differentiate into Schwann cells *in vivo*, do not support the hypothesis that F-cells represent Schwannian/glial differentiation *in vitro*. We postulate that the elimination of amplified N-*myc* gene copies in cultivated NB cells is in line with the phenomenon of tumour cell revertance and that the amplification of oncogenes in malignant cells can be a reversible process leading to the loss of criteria for malignancy.

O-9

IN VITRO DRUG RESISTANCE IS AN IMPORTANT PREDICTOR OF OUTCOME IN ACUTE LYMPHOBLASTIC LEUKEMIA; RESULTS FROM TWO PROSPECTIVE STUDIES

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Objective: To determine the prognostic relevance of *in vitro* drug resistance testing in ALL which could be used for optimal risk-group stratification.

Methods: Drug resistance was measured with the 4-days *in vitro* culture MTT assay on ALL cells obtained at initial diagnosis in two prospective studies: 152 children treated with BFM oriented DCLSG-ALL 7/8 protocol (median follow up 4 yrs) and 141 children of the COALL-92 Study (median follow up 2 yrs).

Results: At risk group stratified analysis in the Dutch study, the *in vitro* resistance to prednisolone, vincristine and l-asparaginase were each significantly ($p<.01$) related to the pDFS or pEFS. Combining the data of these drugs to a resistance profile, showed a 3yr pDFS of 100% for the most sensitive group, 84% for the group with an intermediately sensitive profile, and 43% for the most resistant group ($p<.001$). At multivariate analysis, the resistance profile had prognostic significance superior to and independent from age, tumor burden, phenotype and DNA ploidy.

The second prospective study in the COALL cohort confirmed these results. The 2yrs EFS were 94%, 79% and 47% for respectively the patients with a sensitive, intermediate and resistant profile. When patients from both studies were classified according to the current low-risk (LR) and

high-risk (HR) criteria of the COALL Study group it appeared that drug resistance had strong additional prognostic power. Within the LR group, the EFS was 100%, 80% and 54% for respectively sensitive, intermediate and resistant patients. Within the HR group these figures were 91%, 68% and 56%.
Conclusion: *In vitro* drug resistance is a very strong, independent prognostic factor in ALL with current treatments, that can be used for optimal risk-group stratification. In the new COALL 97 Study children with ALL will be stratified according to their *in vitro* resistance profile.

O-10

TELOMERES DO NOT SHORTEN IN PERIPHERAL BLOOD AFTER ALLOGENEIC TRANSPLANTATION.

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Objective: Telomeres are specialised structures at the end of chromosomes which shorten as cells age. This occurs as telomeres are incompletely replicated during cell division. Continued telomeric shortening is implicated in chromosomal instability and eventual cell senescence. We tested the hypothesis that placing haemopoietic stem cells under extreme proliferative stress such as during intensive chemotherapy programmes or bone marrow transplantation would lead to detectable telomeric shortening or "premature ageing". We examined the telomere length of normal subjects and that of transplant donor/recipient pairs at the same time point following transplantation. **Methods:** DNA was extracted from peripheral blood and digested using the restriction enzymes RSA1 and Hinf1 that do not cut within the telomeric repeating sequence. Digested DNA was separated using agarose gel electrophoresis and telomeres were visualised after direct in-gel hybridisation using an oligonucleotide probe specific for the telomere repeat. Calculation of the Mean Telomere Length (MTL) was performed after exposing the hybridised gel to a PhosphorImager screen and using Image Quant software. VNTR analysis of the donor/recipient pairs was used to assess engraftment. **Results:** The relationship was established between MTL and age for 100 normal individuals (0-96 years). It confirmed a significant loss in telomere length of 27base pairs per year ($p<0.005$). Investigation of 10 transplant pairs (0.5-6 years post transplantation) showed complete donor engraftment in all but no evidence of MTL shortening ($r>0.95$). **Conclusions.** This study confirmed the inverse relationship between MTL and age in haemopoietic cells but was not able to demonstrate that placing haemopoietic cells under replicative stress in BMT also caused telomeric shortening. This suggests that either additional age related factors are important causes of telomeric shortening or that telomere length is preserved in an expanding marrow, perhaps by telomerase expression.

O-11

CIS-ACTIVATION OF THE PROTOONCOGENE *C-FES* BY HIV IN AIDS-ASSOCIATED LYMPHOMAS

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Objective: The study was performed to determine whether HIV integration upstream to *c-fes* was sufficient to cause upregulation of this oncogene, and potentially contribute to cellular transformation.
Methods: The DNA region 5' to the *c-fes* gene transcriptional initiation site was cloned. A luciferase reporter gene was placed 3' to the cloned DNA and attached to the HXB2 and an AIDS T-cell lymphoma LTR at various positions upstream. The constructs were transfected into K562 cells and primary macrophages and tested for modulation of luciferase expression with/without a cotransfected *tat*-containing vector. LTR mutational studies were carried out to determine the role LTR played in *cis*-activation of the luciferase gene.
Summary: Both the HXB2 and lymphoma LTR *cis*-activated expression of the *fes* region of DNA and *tat*-cotransfection further upregulated expression. LTR mutational studies showed that the upregulation was a result of both transcriptional initiation occurring within the LTR and LTR-mediated enhanced expression of the *fes* region. *C-fes* expressed from a CMV promoter expression vector enhanced 3' LTR-mediated *cis*-activation.
Conclusions: This study demonstrates that HIV may be acting as a potential insertional mutagen in a T-cell lymphoma containing an integrated HIV. The data support that the HIV LTR acts as a transcriptional initiation site and as an enhancer element. These observations suggest that in certain circumstances, HIV may act as a directly transforming retrovirus and an extension of this observation would urge caution in the use of retroviral vectors for inserting genes for proposed gene therapy studies. The case of attenuated HIV for vaccine purposes should also be cautiously investigated, given these observations.

O-12

IS THE INTEGRATION OF THE VIRAL GENOME AN IMPORTANT STEP IN THE PATHOGENESIS OF EBV POSITIVE HODGKIN'S DISEASE?

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Overview: Epstein-Barr virus (EBV), is an etiological agent of acute infectious mononucleosis (IM) and is also strongly associated with some malignancies. Evidence for the possible involvement of EBV in the pathogenesis of Hodgkin's disease (HD) came initially from seroepidemiological studies that demonstrated an increased frequency of HD following IM and by the demonstration of the viral genome in a high proportion of HDs. *Objectives:* Our previous results indicted that the status of the virus, episomal versus integrated, and the type of integration may play a fundamental role in the immortalisation processes of lymphoblastoid cell lines (LCL) as we consistently found the EBV in integrated form at specific loci, whereas episomal copies were only found in a sub-population of cells. Up to now, it was believed that EBV is solely present in episomes in HD. This assumption was based on Southern blot analysis. To elucidate the EBV status in HD and IM, episomal versus integrated, we compared *in situ* hybridisation (ISH) and Southern blot results from 21 EBV positive HDs and 6 IMs. *Results:* In lymphoid tissue from patients with IM a low number of cells was positive for EBV and displayed predominantly large diffuse viral ISH signals. In contrast, all Hodgkin and Reed-Sternberg (H/RS) cells in the samples investigated showed small and distinct hybridisation signals, identical to the type of signal seen in interphase nuclei of LCLs with proven viral integration. The number of hybridisation spots ranged from three to nearly one hundred. *Conclusion:* The large diffuse hybridisation pattern found in IM cells indicates an episomal occurrence of this virus. The fact, however, that in HD the signals were found as small distinct dots and sometimes also found in duplicate indicates integrated viruses in the host genome of H/SR cells. Our results suggest that stable integration of the viral genome might represent an important step in the pathogenesis of EBV positive HDs, resembling mechanisms found in other DNA virus induced diseases.

O-13

GENETIC VARIATIONS OF LATENT MEMBRANE PROTEIN 1 OF EPSTEIN-BARR VIRUS IN BURKITT'S LYMPHOMA OF TURKISH CHILDREN

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Burkitt's Lymphoma (BL) in Turkish Children is commonly associated with Epstein-Barr virus (EBV) infection. The C-terminus of the latent membrane protein 1 (LMP1) of EBV is essential for transformation. To understand the molecular mechanism underlying EBV pathogenesis in Turkish children with BL, we analyzed the 30-bp deletion region of the LMP1 gene from paraffin-embedded tumor tissues of 28 BL patients (mean age 5.9 years). Primer pairs spanning the 30-bp deletion region were designed for amplification by polymerase chain reaction (PCR). The PCR-amplified products were analzed by gel electrophoresis, Southern blot hybridization, and DNA sequencing. When compared with the B95-8 strain, 17 (60.7%) of 28 cases had the deletion, 9 (32.2%) or 28 had no deletion, and 2 (7.1%) of 28 had co-infection by two EBV variants, one with and the other without the deletion. Of the 19 isolates with the deletion, variable sizes of deletion were found: 2 (10.5%) had a 30-bp deletion, 14 (73.7%) had a 69-bp deletion, and 3 (15.8%) had a deletion size between 30 bp and 69 bp. To assess the EBV genotype with the changes in C-terminus of LMP1 gene, we performed genotyping by PCR, analyzing EBNA-3C gene locus which can differentiate type A and B strain. All 28 patients were infected by type A EBV, similar to Chinese nasopharyngeal carcinoma which harbored type A virus predominately with a 30-bp deletion. High frequency of larger size (69 bp) deletion has never been reported. Turkish children infected by this from of EBV, in conjunction with other factors, may have increase risk for developing BL.

O-14

TREATMENT (Tt) OF SECRETING INTRACRANIAL GERM CELL TUMORS (S - GCT) WITH CARBOPLATIN (CBP) BASED CHEMOTHERAPY (CT) AND FOCAL IRRADIATION (RT)

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After failure of a Tt based on CT alone, the SFOP protocol for non metastatic S - GCT included focal RT (55 gy) after 3 or 4 cycles of the same CT (CBP 600 mg/m² d1, Etoposide (VP) 150 mg/m² d1-d3, Ifosfamide 1.8 g/m² d21-d25, VP 150 mg/m² d21- d23). In case of biological partial remission (PR), Cis Platinum was introduced. Removal of a residual tumor (T) was recommended before RT.
22 pts (med age 13 years), were registered from June 1992 to September 1996. 4 had suprasellar T, 16 pineal and 2 bifocal. 5 T secreted βHCG, 12 AFP and 5 both. 5 pts had initial surgery (S) and 3 stereotactic biopsy. 1 pt died early after S and 1 is under initial Tt. The 20 evaluable pts achieved biological complete remission (CR) with CT (2 with Cis Platinum). 3 pts had S during CT and 9 at the end of CT with no malignant component found. 1 pt had a marker reevolution before RT and died ultimately with spinal metastasis. 4 pts relapsed in spinal (3) or frontal area (1) but are in CR2 4, 7, 18, 22 m after relapse with Tt including high dose (HD) CT (VP-Thiotépa). 1 pt in CR developed myelodysplasic syndrom and died. 14 patients are in first CR with a follow-up of 6 to 49 m. The EFS is 57% +/- 25% and the overall survival is 76% +/- 25% with a plateau respectively at 18 and 21 m.
In conclusion EFS is improved compared to our previous series of pts treated with CT alone. Focal RT after initial CT prevented local relapses. Metastatic relapses could be successfully treated with 2nd line Tt including HDCT. (Part of this work has been supported by the «Association pour la recherche contre le cancer»).

O-15

PEI IN CHILDREN AND ADOLESCENTS WITH MALIGNANT NON-TESTICULAR GERM CELL TUMORS (mNT GCTs) - RESULTS OF THE GPOH MAKEI 95 STUDY

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Background: Platinum, etoposide and ifosfamide (PEI) are proven to be a very effective combination in high risk malignant testicular primaries in adults. In 1994 the German Pediatric Germ Cell Tumor Group decided to use this regimen to treat patients (pts) with mNT GCTs after incomplete resection or bulky disease.
Patients and methods: From 10/93 to 09/95 45 children were treated as protocol pts with the PEI-regimen. Treatment consisted of cisplatin 20 mg/m² day 1-5, VP16 100 mg/m² day 1-3 and ifosfamide 1.5 g/m² day 1-5. 21 of these pts have received incomplete resection prior to chemotherapy. In 24 children PEI was applicated as neoadjuvant chemotherapy after clinical diagnosis. 2 to 4 courses PEI were administered in total regarding to tumor stage.
Results: 39/45 pts are alive in complete continuous remission (CCR). The overall survival is 71±14%. In detail 7/8 pts with localized mNT GCT and 2 courses PEI after incomplete resection are in CCR. One girl developed AML and died. 13/16 pts with bulky disease and/or metastases, who received 4 courses PEI, are in remission. One child developed a brain metastasis, 1 pt did not respond to chemotherapy and one girl achieved only partial response. 24/26 pts with neoadjuvant treatment and bulky disease are in CCR. Two pts died of disease not responding to chemotherapy. The main reported toxicity was transient leucopenia WHO-grade 3 and 4 in 58% of the pts after 4 courses PEI. Severe infections or nephrotoxicity were not observed.
Conclusions: In respect to previous studies the EFS is comparable instead of shortened treatment duration and reduced toxicity.
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O-16

OPTIC NERVE INVASION IN RETINOBLASTOMA. RESULTS OF TREATMENT WITH TWO SUCCESSIVE PROSPECTIVE PROTOCOLS

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Optic nerve invasion in retinoblastoma is a risk factor for relapse and adequate therapy is not well established.
Patients and methods: From Jun 1987 to Dec 1996, 156 eligible patients with retinoblastoma were enrolled onto two successive protocols (median follow-up 56 months). Of these, 64 had optic nerve invasion without distant or CNS metastasis. All were prospectively classified according to optic nerve, choroid and scleral invasion.
Results: Patients were grouped according to the degree of optic nerve invasion:

1)Prelaminar optic nerve invasion: 22 patients were treated without any adjuvant therapy, 13 of them had concomitant choroid invasion. All survive disease free, except for one case who had progressive disease in the fellow eye and refused therapy and died with progressive disease. 2)Postlaminar optic nerve invasion (resection line free of tumor): 11 were included in our first study (JCO,14:1532,1996) and received 54 weeks of adjuvant VCR/ADR/CPM and no radiotherapy. (pEFS=0.82). In the second protocol, no adjuvant therapy was given provided there was no full choroid or scleral invasion (n=11). pEFS was 100%. No patient with full choroid or scleral invasion was included. 3)Postlaminar optic nerve invasion (resection line with tumor): 14 patients were included in the first study and received 54 weeks of adjuvant VCR/ADR/CPM, intrathecal chemotherapy and 45 Gy orbital radiation. pEFS 0.78% . In the second study, patients with concomitant full choroid and/or scleral invasion (n=6) received an intensified protocol consisting of a total of 8 cycles alternating Carboplatin/VP16 and higher dose CPM/VCR and Idarubicin along with orbital radiaton (45 Gy) and no intrathecal therapy. pEFS was 100%. No patient without scleral or full choroid invasion was included.

Conclusions: Patients with prelaminar invasion and postlaminar invasion with resection line free of tumor and no full choroid or scleral invasion may not need any adjuvant therapy. An intensified and brief chemotherapy with orbital radiotherapy regimen without intrathecal therapy appears to be effective for patients with postlaminar optic nerve invasion with tumor beyond the cut end.

O-17

STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIAS AND NON-HODGKIN'S LYMPHOMA

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Stem cell transplantations for patients with 23 ALL, 26 ANLL and 12 NHL performed in our institute, between March 1988 and December 1996, were retrospectively analyzed. patients : Allogenic BMT from a HLA-matched sibling, if available, was indicated for high-risk ALL in 1st CR or standard ALL in 2nd CR, and ANLL in 1st CR. For high-risk patients without a HLA-matched sibling, auto-PBSCT or BMT from an unrelated donor or a HLA-mismatched family donor was performed. Also, NHL patients in advanced stages took auto-PBSCT. Non-TBI regimen (BVA/BVL:BU+VP-16+ACNU/L-PAM) was used for auto-PBSCT and most of allo-BMT from HLA-matched family donor. For extremely high-risk or refractory cases, TBI-containing regimens were used. result : Event-free survival of each group was as follows.: ALL in 1st CR (4 auto, 6 allo), 90% at 5 years (med. 27 months); ALL in 2nd CR (1 auto, 9 allo), 86% (33 mon.); ANLL in 1st CR (10 auto, 6 allo), 52% (22 mon.); NHL in 1st CR (8 auto), 100% at 3 years (13 mon.). Only 3 patients are alive among ANLL cases in 2nd CR or refractory phase (3 auto, 7 allo). conclusion : 1) BVA/BVL regimen, which is less toxic for children, had adequate antileukemic effects as well as TBI containing regimens, 2) auto-PBSCT for leukemias and lyomphoma seemed to be more effective than conventional chemotherapy in this study, 3) at this moment, unrelated-BMT should be the 1st choice of the treatment for extremely high-risk leukemias such as M0/M6 types, ALL and ANLL showing the karyotype abnormalities with poor prognosis, in case a family donor is not available. 4) Considering the recent advances in the chemotherapy, transplant may be avoidable for NHL, at least, in 1st CR.

O-18

MATCHED RELATED ALLOGENEIC BMT (ALBMT) IN FIRST REMISSION (CR) OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) WITH ULTRA HIGH RISK FEATURES

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The CCG experience in children with ALL in 1st CR identified a small set with <40% 5-yr EFS. In February 1993, CCG initiated an ALBMT study (CCG-1921). Eligibility included any one or more of the following ultra high risk features (UHRF): t(9;22) (q34;q11); *bcr-abl*; t(4;11) (q21;q23) or 11q23; ≤44 chromosomes; infant ALL (2-12 mo) with one of the following: CALLA$^-$ (CD10), WBC ≥100K/mm^3, or day 14 M2 or M3 BM; age ≥10 yrs and WBC ≥200K/mm^3, or delayed CR. Myeloablative therapy included 1200 cGy TBI (200 cGy BID x 3), CTX 60 mg/kg x 2, MTX (day +1, +3, +6, +11), and CSP for GvHD prophylaxis. 40 patients met the eligibility criteria and had a matched (6/6 or 5/6 serology) sibling/parental donor. 27 underwent ALBMT and 13 were treated with alternative therapy. The demographic data between the ALBMT and non-BMT group included median age 105 mo (5–213 mo) vs. 141 mo (19–236 mo), M/F 19/8 vs. 8/5. There was no significant difference in UHRF, except 5/27 vs. 0/13 were infants in the ALBMT vs. non-BMT groups. The 2-yr EFS was significantly better in the ALBMT vs. non-BMT group (56±11 vs. 30±13, p<0.04). The 2-yr EFS for patients with any one of the cytogenetic UHRF was also significantly better in the ALBMT vs. non-BMT group (50±13 vs. 0, p<0.0005). The non-infant UHRF group also had a significantly increased 2-yr EFS in the ALBMT vs. non-BMT group (64±13 vs. 31±13, p<0.01). In the ALBMT group, the incidence of grade II-IV GvHD was 19%, severe VOD was 4%, and the 100-day non-relapse mortality rate was 4%. The relapse rate at 2 yrs post ALBMT was 41%. This pilot study suggests that matched related sibling/parental ALBMT may offer increased survival in a subset of children with UHRF with ALL, especially those with cytogenetic abnormalities.

O-19

STAGE IV NEUROBLASTOMA (NB) OVER 1 YEAR (y) OF AGE AUTOGRAFTED : THE COMBINATION USED IN THE CONDITIONING REGIMEN (Reg) IS THE MAJOR PROGNOSTIC FACTOR (pr-fa).

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High-dose chemotherapy (HDC) is widely used as consolidation of stage IV NB. The results of this approach, although improved as compared to conventional treatment, remain disapointing with a 5 y EFS of about 30 %. Therefore several studies have been performed to define prognostic factors (pr fa). Young age and CR before T have been shown to be favorable pr-fa. However, until now, all studies were performed with multicentric registry data. During the 15 last y 213 NB have been grafted in our pediatric transplantation (T) unit. The disease work up as well as the supportive care techniques were homogeneous along the total period. Therefore we decide to analyse pr-fa in this monocentric series. Reg never included TBI and consisted of polychemotherapy including most often Melphalan (M) and, for 55 % of pts, Busulfan (Bu) combined with M ± Cyclophosphamide.. According to EFS, pr-fa were studied by univariate and multivariate analyses.Factors related to the pt, the disease at diagnosis, the previous treatment, the status at T, and the

type of Reg were studied. In univariate analysis 4 factors were significantly correlated with poor EFS : age > 2 y, BM involvement at diag ; measurable disease post T and Reg without Bu M. In the multivariate analysis 2 adverse factors remain independantly significative : Reg without Bu M and age > 2. In conclusion this monocentric study confirmed pr fa previously established (age) and demonstrated the superiority of Reg containing Bu M over other polychemotherapy combinations tested.

O-20

TUMOR CELL CONTAMINATION OF PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) IN PATIENTS WITH EWING TUMOR

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PBPC autografting after high-dose chemotherapy is increasingly used in the treatment of patients (pts) with high-risk Ewing tumor (ET). Currently it is not clear, if tumor cell contamination of autografts may contribute to systemic relapse. In this prospective, single-institution, blinded study we investigated aliquots of PBPC harvests for the presence of ET characteristic EWS chimeric RNA by RT-PCR. So far, 25 PBPC from 12 pts were investigated: 6 pts had isolated lung metastases, 4 had bone/bone marrow +/- lung metastases and 2 pts had local recurrence of a pelvic tumor. At diagnosis, PCR in the bone marrow was positive in 8/12 pts. At PBPC harvest all patients tested were PCR negative in the bone marrow. All collections of PBPC were performed before definitive surgery or irradiation of the primary tumor. PBPC harvests were PCR positive in 4 pts (33%). 11/12 pts received megatherapy with PBPC reinfusion (5 pts in CR, 2 in VGPR, 4 in PR) 3 of these 11 pts received positive PBPC reinfusions (all 3 in PR at harvest). So far, 4 pts relapsed systemically (3 with PCR positive PBPC reinfusion and 1 with negative reinfusion). 7/8 pts who received PCR negative reinfusions are still in remission after a median follow up of 15 months. In summary, our preliminary results suggest that tumor cell contamination of PBPC might contribute to relapse. However, these data need confirmation on a larger number of patients with high-risk ET.

O-21

RESULTS OF A PEDIATRIC ONCOLOGY GROUP PHASE III TRIAL COMPARING CONVENTIONAL VS. HYPER-FRACTIONATED RADIOTHERAPY IN CHILDREN WITH BRAINSTEM GLIOMA (BSG)

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Purposes/Objective: In June 1992, POG began accrual to a Phase III study, POG 9239, designed to compare the time to disease progression, overall survival, and toxicities observed in children with newly diagnosed BSG (arising in the pons) treated with of infusional Cisplatin and randomized to either conventional or hyperfractionated radiotherapy (RT). The trial was closed in March 1996, having achieved its accrual goal.

Materials & Methods: Treatment (Rx) consisted of a six-week course of local field RT with either once a day treatment (Rx 1) of 180 cGy per fraction to a total dose of 5400 cGy or a twice a day regimen (Rx 2) of 117 cGy per fraction to a total dose of 7020 cGy, with 100 mg/m^2 of Cisplatin delivered by continuous infusion over a 120-hour period, beginning on the first day of RT on Weeks 1, 3, and 5. Of the 132 pts accrued to the study, 94 pts were eligible for analysis at this time, 47 in each Rx arm. As of 4/18/96, the study coordinator had not yet verified eligibility and assessed the evaluability of the remaining pts.

Results: All results are from time of diagnosis through April 1996. The median time to disease progression and to death were 5.5 mo and 8 mo for pts treated on Rx 1, and 5 mo and 8.5 mo for pts on Rx 2, respectively. The 1 yr and 2 yr survival rates were 29.1% and 9.0% for Rx 1 and 23.6% and 6.5%, respectively.

Conclusions: The hyperfractionated method of Rx 2 did not improve survival (p=.44) over that of the conventional fractionation regimen of Rx 1. Both treatments are associated with a poor disease-free and survival outcome.

O-22

CHILDHOOD EPENDIMOMA (EPD). PRELIMINARY RESULTS OF A STUDY OF THE ITALIAN ASSOCIATION OF PEDIATRIC HAEMATOLOGY ONCOLOGY (AIEOP)

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Objective. Principle objectives of the study are: 1) to stage and treat homogeneously the largest possible population of children affected by EPD; 2) to investigate the role of *a)* VCR, CTX and VP16 followed by high dose hyperfractionated radiotherapy (HF-RT) in improving disease-free survival in children with evidence of disease (ED) after surgery (S), and *b)* of HF-RT alone in those with no ED (NED) after S. Methods. Patients (pts) were staged with 72-hour post-s Gad enhanced head and spine MR. The entity of the surgical act was codified according to the SIOP Brain Tumor criteria (MPO'94). Pts NED were submitted to involved (plus 2 cm margin) field 2 fractions/d RT (1.1 Gy x 2/d, total dose 70.4 Gy); the ones with ED were to receive four monthly cycles of VEC CT (VCR 1.5 mg/sqm weekly cycle 1 and 3; VP16 100 mg/sqm d 1,2,3; CTX 3g/sqm d1) followed by the same RT. Elegibility criteria. Aged 3-17, no previous treatment, histologic diagnosis and complete tumor investigation (pre- and post-S head+spine MR plus CSF cytology). Results. From 9/ '93 to 1/ '97, 38 children have been enrolled (24 M, 14 F, median age 6 yrs), 7 EPD were supratentorial, 31 arose in posterior fossa: 31 pts were elegible, 4 were at relapse after S only, 3 infants (<3 yrs) were considered on study and submitted to 6 cycles of CT omitting RT. 22/31 eligible pts had been completely operated, 9 were ED after S. 19/22 (86%) pts with complete S were alive NED (median f-up 10 mos); 3 relapsed: 1 locally, 1 in the spine, 1 locally and in the spine. 5/9 pts with incomplete S were alive NED (median f-up 15 mos), 4 progressed locally. In 5 evaluable pts CT obtained 1 CR, 2 PR and 2 SD. 2/4 pts treated at relapse were alive NED at 13,18 mos from relapse; 2 were alive with SD. 1/3 infants was alive NED 28 mos from diagnosis, 2 had local relapse and died. Toxicity of CT was unremarkable, while S was followed by major neurological sequelae that induced to postpone (6) or give up (1) to complementary treatment. As to feasibility, double fraction RT was not performed in 6/31 cases. Conclusions. Pts accrual was satisfying (11/yr). Feasibility and compliance of treatment were low, especially because of severe post-surgical neurologic conditions and poor PS of pts. F-up is too short to consider reliable treatment results. The favourable outcome of pts NED after S treated with local RT seems to be confirmed. Role of daily bi-fractionated RT and VEC CT still needs to be explored.

O-23

RESULTS OF THE FIRST UKCCSG/SIOP STUDY OF PRIMARY CHEMOTHERAPY FOR TREATMENT OF INTRACRANIAL PRIMITIVE NEUROECTODERMAL TUMOURS (PNET) IN CHILDREN UNDER 3 YEARS.

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In 1992 the United Kingdom Children's Cancer Study Group (UKCCSG), opened a protocol investigating a multi-agent chemotherapy schedule for children under 3

years of age with a malignant brain tumour. Treatment was designed to alternate myelosuppressive drugs (carboplatin Day 1 and cyclophosphamide Day 28), with less myelosuppressive agents administered at white count nadir (high dose methotrexate Day 14 and cisplatin Day 42) in the hope of preventing early emergence of drug resistance, and to delay radiotherapy for at least one year. Radiotherapy was not undertaken unless there was evidence of disease progression. By September 1996 36 children with a diagnosis of intracranial PNET had been registered and 34 had been treated on the chemotherapy schedule. There was a slight excess of male children (21 boys to 15 girls) who were enrolled at a median age of 21months. The majority of primary tumours arose in the posterior fossa (75%) and between 20% (if only pre-operative imaging detected a true positive) and 50% of patients had established metastatic disease at diagnosis. At a median follow up of 10 months (maximum 41 months) the 2 year event free survival (EFS) of treated patients is 4% (C.I.< 1% - 19%). 27 patients progressed on the study with a median time to progression of 6 months. The pattern of progression varied and was not related to the initial distribution of disease at presentation. Overall survival (OS) was influenced by the decision to irradiate, which was only undertaken in 50% of patients. This was in turn possibly influenced by age at relapse (median age at progression in the irradiated group 38months, compared to 20 months in the non-irradiated group, p=0.01). Proportionately more patients with leptomeningeal progression went on to receive salvage radiotherapy, and the OS of the total patient group is 27% (C.I. 13 - 47%) with only 1 patient surviving in the long term without irradiation. **Conclusions:** Despite a relatively intensive (although not dose intensive) schedule, chemotherapy did not prevent rapid disease progression, either at primary or metastatic sites. Some children may have been salvaged by radiotherapy, although this was only performed in 50% of patients, reflecting the deep reluctance to use radiotherapy in young children. In view of the poor EFS the study has been closed to PNET's, although remains open for young children with all other malignant brain tumours.

O-24

RANDOMISED CLINICAL TRIAL OF PRE RADIOTHERAPY CHEMOTHERAPY VS RADIOTHERAPY ALONE FOR MEDULLOBLASTOMA (PNET III)

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The SIOP trial PNET III managed on behalf of SIOP by UKCCSG is a randomised study for children over age 3 and under age 16 with non metastatic medulloblastoma. The study questions whether or not pre-radiotherapy chemotherapy improves disease free survival over radio-therapy alone. The study remains open at the present time and this is an interim report. Patients and Methods: In a European Multicentre trial patients with non metastatic medulloblastoma as evidenced by MRI scanning are treated with surgery to obtain maximal surgical removal and are then randomised to receive either craniospinal radiotherapy (35Gy to craniospinal field, 55Gy to tumour) or to receive chemotherapy with Vincristine Carboplatin and Cyclophosphamide for 70 days before commencing radiotherapy at day 100. Centres are asked to notify all patients registered and to indicate reasons for non-randomisation. Results to Date: 144 patients have been randomised, 164 have been notified but not randomised. In the randomised patients there have been 25 tumour progressions and no toxic deaths. Chemotherapy data is available on 48 patients. 41 of 48 received full protocol dose. 46 of 48 experienced grade III or IV haematological toxicity. 12 of 48 experienced grade III or IV gastrointestinal toxicity. 1 child had tumour progression during chemotherapy. No child progressed during radiotherapy. The non randomised patients included 34 patients with known metastatic disease. They were all treated with chemotherapy. To date 14 have had disease progression. 130 patients were not randomised either because of parental refusal or clinician choice of whom 43 have progressed. Median follow up is 17 months. Conclusion: No significant differences are yet emerging between the two therapies. Accrual to the trial is slow and needs to be improved, the number of patients excluded by clinician choice is too high. The results in the randomised group are significantly better than in the non randomised group and analysis will be conducted to exclude selection bias in patients entered into the trial.

O-25

NEUROBLASTOMA (NB) AND OPSOCLONUS-MYOCLONUS SYNDROME (OMS). THE ITALIAN EXPERIENCE IN A 16-YEAR PERIOD.

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OMS (opsoclonus, ataxia and polymyoclonia) occurs in only 1-2% of NBs. We report on a new series of 15 pts with this association diagnosed between 1979-1995 (1.3% of all newly diagnosed NBs registered in the same period). Nine were male and 6 female. Median age was 30 mos (range 3-75). Thirteen had localised (INSS stage 1-3), one widespread (stage 4) and one plurifocal (stage 4s) disease. Histology (Shimada classification) was unfavourable in 3/15. Eleven tumors showed marked lymphoid infiltration. Lymphocytes were studied immunohistochemically in 5 and always found CD20+ and HLA-DR+. None of the 15 tumours evaluated for MYCN had amplification of the gene. Two of 15 had diploid DNA content. OMS was present at diagnosis of NB in 13 pts while preceded it in 2 (of 3 and 6 mos, respectively). Thirteen of 15 pts survive at 10-145 mos (median 84). Two died of disease with 5-y overall survival 84% and event-free survival 76%. Of 13 survivors 7 had neurologic recovery in 2-4 mos after tumor removal without specific treatment. The other 6 received steroids and/or ACTH and more recently steroids plus intravenous immunoglobulins. Despite these treatments 2/6 have neurologic sequaele including cognitive-motor delay and speech disturbances. Our data confirm that children with NB and OMS have overall a good prognosis possibly because they usually present with favourable clinical, histological and biological features. In our experience OMS regressed or persisted independently of "specific" therapies. Those with incomplete regression of OMS may eventually develop severe neurologic abnormalities requiring early detection, careful monitoring and proper treatment.

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O-26

INTERNATIONAL NEUROBLASTOMA 'RISK GROUPS' - A PRELIMINARY REPORT FROM THE WORKING PARTY

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There are many 'prognostic variables' in neuroblastoma (Nbl) but no consensus, yet, as to which combination of these variables gives the most useful clinical information. The **Objective** of the International Neuroblastoma Risk Group (INRG) Working Party - which succeeded the INSS Group and met in Berkeley, UK, in 1995 - was to develop a pragmatic risk group system usable by the entire paediatric oncology community to stratify patients (pts) in treatment trials and thereby compare results.

Methods
Data on 2,832 pts registered in French, German, Italian, Spanish, ENSG, POG and CCG studies were combined and centralised at the Univ. of Florida. Many of these retrospective data sets were incomplete but there was sufficient information, using the 5 most commonly available variables (age, Evans stage, MYCN, Ferritin, and LDH) and recursive partitioning analysis, to divide pts into four groups (A, B, C and D) with significantly differing prognoses. Subgroups of the Working Party have, respectively, developed a histopathology classification system (INHC) and recommendations for sampling and prioritisation of tests on tumour samples.

Conclusion and Summary
The 'building blocks' of an INRG system are now in place but require prospective study in clinical trials, with international co-

operation, so that the system can be validated and refined. When finalised, INRG will provide a much-needed 'common language' for Nbl management.

Financial support was provided by the Forbeck Foundation, the Neuroblastoma Society (UK), & RICC (Gibraltar).

O-27

SOMATOSTATIN RECEPTORS *IN VIVO* ARE PREFERENTIALLY EXPRESSED IN TRIPLOD NEUROBLASTOMAS BUT ABSENT IN 1P-DELETED TUMOURS.

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Introduction: Unfavourable neuroblastomas are often correlated with deletion of chromosome 1p36, whereas favourable tumours often have triploid DNA content . Somatostatin receptors (SR) have recently been characterized in neuroendocrine cells and related tumours e.g. neuroblastomas. Five subtypes of the SR have been cloned. Long acting somatostatin analogues (e.g. pentetreotide) bind to SR with high affinity and may be used for *in vivo* detection of SR and tumours with SR expression.
Methods: We examined 45 children with neuroblastoma at diagnosis/relapse for SR expression *in vivo* by ^{111}In-pentetreotide scintigraphy. MIBG scintigraphy was performed in all children. Tumour tissue was investigated for aberrations of chromosome 1p using FISH and/or PCR-based microsatellites, and DNA content with flow cytometry and/or image cytometry.
Results: SR expression was shown in 27/45 tumours. Scintigraphic tumour detection was less sensitive with ^{111}In-pentetrotide than mIBG (60% vs. 93%, p=0.058). Thirteen tumours were deleted at 1p36, none of these 1p-deleted tumours showed SR expression (p<0.001). 21/45 tumours had di/tetraploid DNA-content whereas 24 were triploid (DNA-Index: 1.25-1.75), 22/24 triploid tumours showed SR expression (92%, p<0.001).
Conclusions: Somatostatin receptor expression in neuroblastoma is highly correlated with tumour biology. SR scintigraphy (in combination with mIBG scintigraphy) may be a parameter of prognostic relevance. Somatostatin receptor scintigraphy at diagnosis may offer minimal-invasive early prognostic information with special relevance to early detected tumours and the decision for treatment.

O-28

A NEW IMMUNOTHERAPY AGAINST NEUROBLASTOMA (NB) BASED ON NATURAL HUMAN ANTI-NB-IGM - ANTIBODIES

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A novel cytotoxic mechanism against NB cells based on natural immunoglobulin M serum antibodies has been used to establish a new immunotherapy. The occurence of these antibodies which are able to induce complement-mediated cytolysis as well as apoptosis of NB cells in vitro and in vivo has been investigated in several age groups of healthy persons and in patients with NB. Whereas in neuroblastoma patients no anti-neuroblastoma-IgM-antibodies could be found the prevalence of these antibodies in healthy individuals of different age groups were as follows: 0-1 yr 5%; 1-4 yrs 65%, 5-14 yrs 82%, 15-19 yrs 51%, 60-100 yrs 28%. To determine the cytotoxicity of individual serum samples the following method was used: Aliquots of 4 x 10^5 LA-N-1 NB cells were incubated with 100 µl of human serum. After incubation for 1 hr at O° C 200 µl of a standard complement serum was added. Following reincubation for 45 min at 37° C the percentage of cell death was analyzed by propidium iodide fluorescence using a FACScan. By this method standard blood donors were screened in order to select individuals with a high (i.e.> 80%) serum cytotoxicity against NB. Using those sera a phase I/II study in patients with relapsed NB (INNS stage 4) has been instituted based on a complete plasma exchange by plasmapheresis (plasma separator Spektra, Cobe Comp.).

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O-29

**NEPHROBLASTOMATOSIS :
A PEJORATIVE FACTOR IN WILMS TUMOUR**

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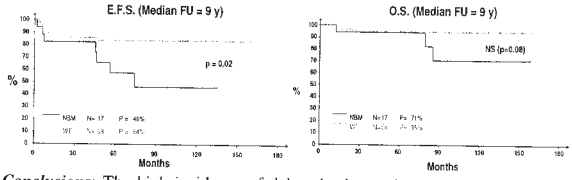
The Nephrogenic Rests (NRs)(abnormal persistent metanephric cells), may be localised diffuse or multifocal in one or both kidneys and are defined as Nephroblastomatosis (Nbm). The aim of this retrospective study is to investigate the prognosis of Wilms tumour (Wt) associated or not with Nbm.
Patients and Methods: From 1983 to 1993, 75 pts with Wt were treated in Centre Léon Bérard. At diagnosis 58/75 (35f, 23m) had Wt only (Wt grp) and 17/75 (7f, 10m) had Wt with Nbm (Nbm grp). The mean age (mths) was 44 in Wt grp, and 40 in Nbm grp. In 3/17 pts (f) the Nbm was found first one at 18,11 and 10 mths of age and was treated by Vincristin and Dactinomycin, and when Wt appeared by SIOP protocol. The treatment was according to the SIOP 6 (n=45) and SIOP 9 (n=30) protocols.
Results: The Nbm histological subtype distribution was perilobar-NRs in 12 pts (8f, 4m), intralobar-NRs in 3pts (1f, 2m) and combined type in 2 pts (1f, 1m). The lesions were diffuse in 6/17. The Wt stage(st) distribution was 32 st I, 15 st II, 7 st III, 10 st IV and 11 stV [8 in Nbm grp (synchronous in 5 cases, metachronous in 3 cases) and 3 in Wt grp]. Anatomopatological staging of Wt in Nbm grp was:10 st I, 6 st II, 1 st III. The onset of the second tumour in stV metachronous with Nbm occured later (62, 44, 30 mths) than in Wt cases (5, 8mths). 7/17 pts relapsed in Nbm grp versus 9/58 in Wt grp. The EFS of Nbm and Wt grp were 46% and 84% respectively (p= .02) and the OS were 71% and 95% respectively (p= .08).



Conclusions: The high incidence of delayed relapses in Wt with Nbm justify a long term (over 10 years) follow-up with intensive survey during the first 5 years. In our series Nbm seems to be associated with an unfavourable prognosis of Wt. Treatment of Wt with Nbm should be more intensive or prolonged and not necessary related to Wilms anatomopathological stage after surgery. (Supported by la Ligue Contre le Cancer, comité départemental du Rhône).

O-30

IMPORTANCE OF THE INTRAABDOMINAL TUMOUR EXTENSION AT SURGERY FOR RENAL TUMOURS IN PATIENTS YOUNGER THAN 6 MONTHS. THE SIOP NEPHROBLASTOMA STUDY No-9.

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The SIOP-9 study on Wilms' tumour was focused on pts aged > 6 m, however,

those younger were also included. In this group primary nephrectomy was advised whenever possible because of high rate of benign tumours and elevated risk of chemotherapy (CT). **Aim:** to evaluate the importance of the intraabdominal extension of renal tumour (RT) in a group of young pts with relatively favourable prognosis. **Patients:** 42 unilateral non-metastatic pts younger than 6 m were registered (1987-1991: 5% of all 852). **Treatment:** Primary nephrectomy was undertaken in 37 pts (88% of all; age 0-6 m; RT volume m=163 ml). Preoperative CT was given to 5 pts, in whom primary surgery seemed difficult (12% of all: age 3-6 m, initial RT volume m=495 ml). **Stages:** I/61%, IIN-/13%, IIN+ & III/26%. **Pathology:** fibroadenomatous (1pt) & mesoblastic nephroma (MN; 19 pts) - 48%, standard (19 pts) - 45%, clear cell sarcoma (1 pt) & malignant rhabdoid tumour (MRT; 2 pts) - 7%. RT ruptures were observed only in not pretreated pts (6=16% of primarily operated on; 5 MN). Also the extrarenal extension was observed only in pts primarily operated on (4 pts = 11%): 3 were MN and 1 was MRT. The extrarenal involvement was multiple in 3 of 4 pts and concerned the perirenal fat in all, liver in 2, v. cava in 2, renal v. in 1 and adrenal gland in 1. These extensions were completely resected in 3 pts (CR), but not - in 1 (relapsed, MN). Regional lymphnodes (LN) involvement was suspected in 6 pts and microscopically confirmed in 2 of them. LN were radically excised in 1 pt (standard RT, alive), but not - in the other (standard, ruptured, died). **Outcome:** 4 relapses occurred: 3 in MN (2 stage III; 1 stage I) and 1 in MRT (stage III). Two pts died of RT (MN and MRT) and 2 are in IInd CR (both MN). OS and EFS at 5 y were 95% and 91%. **Summary:** Favourable outcome in young pts was confirmed. Rare relapses concerned also MN (3 of 4 cases). Extrarenal tumour extension did not not imply poor prognosis if completely resected. RT rupture had little influence on prognosis in MN (of 5 ruptures in these pts, 1 was followed by relapse). Pretreatment assures uneventful surgery and may be useful in pts aged > 3 m with extensive RT, when risk of rupture at primary surgery is high (16%), probability of MN decreases (only 2 of 19 mes.neph. were aged > 3 m), and risk of CT is more acceptable. None of pretreated pts had a benign RT.

O-31

LYMPH NODE POSITIVE SOFT TISSUE SARCOMA (STS) - DESCRIPTION AND IMPACT OF LOCAL LYMPH NODE THERAPY ON OUTCOME AND LYMPH NODE RELAPSE

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Introduction: Lymph node (LN) involvement is globally considered as a very poor prognostic marker in chemosensitive STS (RMS, EES/PNET, SS, UDS). The aim of this study was to investigate the reasons and impact of specific LN therapy on LN relapse.
Patients: Out of 924 paediatric pts. (≤21 yrs., CWS study 81 to 91) with localised (stage I-III) chemotherapysensitive STS we identified **92 pts.** (10%) with proved LN involvement. The median age in this group was 10 yrs. (range 0-19 yrs.) and median follow-up was 40 months. The histologies were: 50 RME, 31 RMA, 7 PNET, 2 UDS, 2 SS. Beside the general outcome we focused on the individual LN treatment and divided arbitrarily between pts. who received mainly radiotherapy (RT), mainly surgery (lymphadenectomy, OP) or „none" of this local LN therapies. Pts. with (diagnostically) LN biopsies or sampling, but no RT were also summarised under „none".
Results: As depicted below the general outcome was quite impaired with 49% pts. in continuos complete remission (CCR) and only 55% alive. Beside this, only 10/92 (10%) pts. with initial LN involvement developed a LN relapse (LNR).

	All				RME				RMA			
LN therapy	All	*RT*	*OP*	*none*	All	*RT*	*OP*	*none*	All	*RT*	*OP*	*none*
n (pts.)	**92**	*39*	*23*	*43*	**49**	*14*	*12*	*27*	**31**	*20*	*8*	*9*
in CCR	**49%**	*38%*	*48%*	*53%*	**57%**	*43%*	*58%*	*63%*	**42%**	*35%*	*38%*	*44%*
alive	**55%**	*49%*	*57%*	*58%*	**61%**	*50%*	*67%*	*67%*	**52%**	*45%*	*50%*	*56%*
LNR	**10%**	*8%*	*7%*	*12%*	**8%**	*7%*	*8%*	*7%*	**19%**	*15%*	*13%*	*33%*

The difference between pts. receiving LN therapy (RT/OP) or not (none) is not dramatic, characterised by nearly identical outcome rates and minimal differences in LNR (p<0,7). In alveolar RMS however, LNR seems to occur more often than in embryonal (p<0,14) and the difference between pts. with vs. without LN therapy seems more obvious (p<0,3)
Conclusion: The minimal differences between the different LN therapies may be interpreted in 3 directions: 1. LN biopsies/sampling (which were summarised under „none") is already a **sufficient** LN treatment. 2. Especially in RME LN may be treated sufficiently by chemotherapy **alone**. 3. There is a difference between the therapy modalities, but our series of 92 pts. is too small to show this **significantly**. In summary the field of LN therapy needs further investigation, especially by establishing a standardised treatment. LN relapse, however is even in initially LN positive STS a very rare event (10%).

O-32

PROGNOSTIC FACTORS IN CHILDREN: WITH METASTATIC RHABDOMYOSARCOMA: RESULTS OF THE EUROPEAN INTERGROUP STUDIES (EIS) MMT'89 AND MMT'91

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The EISMMT-IV, opened to patients entry on May 1989, was closed on August 1996. 161 eligible patients, enrolled into the studies, are evaluable for treatment. The treatment plan has already been described. The major difference between MMT89 and MMT91 protocol was the substitution of the fourth cycle of chemotherapy with high dose Melphalan 200mg\m^2 + autologous bone marrow rescue (ABMT) as consolidation after complete remission (CR).
The 5 year progression free survival (PFS) and overall survival (OS) are 20.8% and 28% respectively (median follow up 32 months).
In order to determine prognostic factors the following pretreatment variables were considered: histology (alveolar vs. non alveolar), age (<10 vs.>=10), tumor invasiveness (T1 vs.T2), bone marrow- bone-lymph node- lung metastasis, regional lymph node involvement (yes vs. no), primary site (grouped into two broad categories) , number of metastasis (1 vs.>1), tumor size (<5cm vs.>5cm) and treatment variables (surgery, radiotherapy, megatherapy)
Survival curves were calculated by the Kaplan-Meir method. The statistical significance of each variable was tested by the logrank test, while the relationship of multiple charateristics to survival was evaluated using the multivariate proportional hazard model of Cox.
Univariate analysis showed that primary site (p=0.0007), age (p=0.0001), bone marrow metastasis (p=0.0001), bone metastasis (p=0.0005), number of metastasis (p=0.0075) and surgery (p=0.0012) were related to survival.
Multivariate analysis confirmed bone marrow metastasis (p=0.05), age (p=0.03), site (p=0.0059) and surgery (p=0.03) as prognostic factors while high dose Melphalan didn't seem to play a role in long term consolidation of patients in CR.
Conclusions : the multivariate analysis select three pretreatment factors that can be taken into account in defining groups of patients with better chance of long term survival. Surgery of residual disease must be considered as important factor in treatment strategy.
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O-33

RENAL TUMORS IN CHILDREN UNDER 12 MONTHS OF AGE

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Objective SIOP studies advocate primary nephrectomy (PN) in patients ≤6 mo of age with a renal tumor, because of the high incidence of benign tumors, and the morbidity of chemotherapy in this age group. Patients aged 7-12 mo are pretreated. Aim of the study: to make an inventory of 1) how these guidelines were followed, 2) the incidence of benign tumors, 3) the treatment given in patients ≤6 mo of age, and 4) to compare treatment and outcome with patients 7-12 mo of age.
Methods Retrospective analysis of 70 patients (1969-1995) under 12 mo of age at diagnosis, treated in three different Centers. Demographics, pathology, staging and treatment variables were identified, survival was determined by Kaplan-Meier analysis.
Results Of 70 patients (44 at EKZ/AMC, 16 at UHN, and 10 at SCH), 41 were males and 29 females. Twenty nine (41.4%) infants were 0-6 mo of age (group A) and 41(58.6%) were 7-12 mo old (group B). *Group A* contained 4 pts (13.7%) with Mesoblastic Nephroma (MN), of whom 1 was still-born. Of the 25 Wilms' tumor (WT) cases, 13 were stage I, 2 stage II, 7 stage III, and 3 stage V, of whom 1 died at diagnosis. *Treatment*: 15/29 (51%) by PN, of whom 8 with surgery only (SO), 19/29 (65.5%) with chemotherapy (CT) and 8/29 (27.6%) with radiotherapy (RT). Tumor rupture occurred in 2 pts (1 PN, 1 pretreated), mets developed in 2 (WT stage III), 2 WT patients had a metachronous tumor. *Group B* contained 1 (2%) MN, 26 WT stage I, 7 stage II, 6 stage III, and 1 stage IV. *Treatment*: 13/41 (31%) by PN, of whom 1 with SO, 39/41 (95.1%) with CT and 9/41 (22%) with RT. Tumor rupture occurred in 1 (PN), mets developed in 5 pts (1 in MN, stage I, II, III, and IV, respectively), 2 WT patients had a metachronous tumor. *Outcome*: All 4 MN patients have NED (mean follow-up time 140 mo, range 21-305 mo). Five year survival in stage I WT was 100% in group A and 95% in group B,

stage II 100% in A and 71% in B, and stage III 68% in A and 66% in group B. The stage IV patient died 78 mo after treatment, 1 stage V patient died 4 mo after treatment, the other has NED after 204 mo of follow-up.

Conclusion Despite the SIOP recommendations, only 51% of pts ≤6 mo were treated with PN, whereas the percentage treated with CT was unexpectedly high (65.5%). Less benign tumors were found (13.7%) than reported in the SIOP studies (20-70%). Survival was equal in patients under and above 6 mo. Regarding the CT treatment given, the future study of late effects becomes most important.

O-34

FOCAL NODULAR HYPERPLASIA IN CHILDHOOD ELEVEN CASES WITH SURGICAL TREATMENT

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Focal nodular hyperplasia (FNH) is a rare benign tumor of liver in childhood. In the case of a solid liver tumor with normal serum AFP value, diagnosis of FNH is suspected because of typical CT-scan and/or MRI features and can be assessed by histological examination. FNH is more frequent in females, and its growth is thought to be related to hormonal therapies or events. Most of authors are reluctant to propose surgery for HNF and favor periodic sonographic supervision. However quality of life of girls submitted to non-operative management is impaired since oral contraceptive therapy is contra-indicated and, above all, pregnancy is not recommended. It is why surgery is presently proposed to these patients in our institution. The aim of this work is to report on a series of 11 children who underwent surgery for FNH.

From 1982 to 1996, eight girls and 3 boys presented with FNH at age 2 to 15 years (mean = 9.5). Eight of them had palpable liver mass and 3 tumors were found by US-scan because of abdominal pain. Size of tumors ranged from 5 to 20 cm (median=9 cm).

Initial non operative management (duration: 2 months to 3 years) was choosen for 4 patients. Isolated or associated indications for surgery were volume or growth of tumor (4 pts), doubt in diagnosis (6 pts), symptoms or abnormal liver tests (5 pts). Preoperative arterial embolization was performed in the 3 first patients and the technique of vascular occlusion was used in the 5 last ones. A complete resection could be achieved in 10 patients. The first patient, a girl, underwent a partial resection at age 13 and demanded at age 20 a successful secondary total resection. Diagnosis of FNH was confirmed in all cases. Post operative course was uneventful in 8 cases. With a follow-up of 1 to 6 years (mean=2.5), all patients are alive in good condition, without evidence of secondary FNH.

We conclude that modern liver surgery may offer safe and radical cure of HNF in children and teen-agers and should be considered especially in girls.

O-35

RESECTION OF NEUROBLASTOMA (NB) IN 31 CHILDREN USING INTRAOPERATIVE SCINTILLATION (ISD) DETECTION WITH I^{125}-MIBG.

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Optimal tumor resection and precise post-surgical staging are necessary to achieve remission of **NB** and to correctly assign following therapy. Surgery with **ISD** may improve local tumor control.

From May 1990 to January 1997, 31 children (5 months to 14-year-old) underwent 35 surgical procedures with ISD. According to Evans staging, there were 19 stage IV, 8 stages III, 3 stages II, and 1 stage I NB. All children were treated according to SFOP protocols with primary surgery (n=3), or primary chemotherapy (n=28). Six patients had already been operated on before and had had additional procedures with ISD for local recurrence or incomplete preliminary resection. Ten µCl/kg of I^{125}-MIBG were injected 3-5 days before surgery. A CdTe hand held γ-detecting probe was used for detection. After surgery, patients were classified according to pTNM staging and treated according to SFOP protocols.

ISD was not helpful in 16 procedures (46%): no evidence of fixation by the tumor (n=6), macroscopic incomplete resections (n=6), technical failures (n=2), per-operative death (n=1), no tumor (n=1). Post operative staging were: 1 pS0, 5 pSI, 3pSIIIa, 4 pSIIIc, 2 pSIV (both pT3c), 1 per-operative death.

ISD was helpful in 19 procedures (54%), confirming complete macroscopic resection of the primary tumor and lymph nodes. In 7 procedures ISD was also useful to localize positive lymph nodes or local reccurences which were not visible. **Histologic examination of these 7 additional resections showed active NB cells**. Post operative staging were: 4 pSI, 13 pSIIIa, 2 pSIV (both pT3a). Follow-up (FU) for this group of patients is: alive with no evidence of disease: CR1 (mean FU=26 months): n=8, CR2 after local (n=1) or metastatic (n=1) recurrence; alive with disease: local recurrence n=1 or persistant metastasis (pSIV) n=1; deceased with local (n=3) or metastatic (n=1) or local + metastatic recurrences (n=2), post operative death n=1.

ISD is helpful to achieve and to confirm complete macroscopic resection of NB.

O-36

FIBROSARCOMA: RESULTS OF THE ITALIAN COOPERATIVE STUDY RMS-88.

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From Genuary 88 to December 95, 17 localized fibrosarcomas, out of 198 NRSTS (8.6%), were registered in the ICS RMS 88: 8 m. 9 f.. Median age: 47 mos. (range 8gg - 177mos.). Primary site: extremities 12 (8 proximal, 4 distal), other 5 (thoraco-abdominal wall 3, lung 1, intraabdominal 1). 16/17 pts., evaluable for treatment, were grouped according to IRS staging system as follows: 10 Gr.I, 3 Gr.II, 3 Gr.III.

Treatment and Results.

GR.I . *5/10* pts, aged 1,6,27,65,124 m., underwent Surgery (S) alone: they are disease free, 4 in 1^ Complete Remission (CR), 1 in 2^ CR after Local Relapse (LR). *4/10*, aged 8,27,101,112 m., had S (in 1 case mutilating) + Chemotherapy (CT) according to IVA regimen: 3 are disease free (1 suffered of a LR) and 1 died because of 2° tumor (appeared 24 months from diagnosis). *1/10*, aged 177 m., who had S+CT (IVA)+Radiotherapy (RT, 41Gy), is in 1^ CR. **Gr.II** *2/3* pts, aged 3 and 7 m., received only S (in both cases microscopical residuals were only suspected): 1 is in 1^CR, 1 was lost to follow-up after LR. *1/3*, aged 24 m., had S+CT (IVA) and died for Progressive Disease (PD) after LR+metastases 23 m. later. **Gr.III**. *2/3* pts, aged 1,7 m., received CT+S (mutilating in 1 case), and are disease free; *1/2* (60m.) had CT+RT(42Gy)+S and is disease free. All of them showed poor response to primary CT (VAIA regimen).

Remarks.

13/16 pts (81%) are disease free (follow-up 24-96 mos.): 11 pts in 1^CR (7 Gr.I, 1 Gr.II, 3 Gr.III), and 2 in 2°CR after LR (Gr.1). 2/16 pts (12.5%) died: 1 pt for PD (Gr.II) and 1 for 2°tumor (Gr.I). 1/16 pt was lost to f.u. after LR. In our experience S has been the mainstay of treatment, while the efficacy of CT must be confirmed also in young pts. LR was the cause of 3/4 treatment failure. Age does not seem to be related to prognosis (CR was achieved and maintained in 6/10 pts aged<5 years vs. 5/6 older pts.).

O-37

HEPATOBLASTOMA WITH PORTAL CAVERNOMA SURGICAL POSSIBILITIES

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Obliteration of the portal vein branch supplying the involved part of liver is a common finding at time of diagnosis of an hepatoblastoma (HB). Neoplastic portal vein thrombosis resulting in portal cavernoma (PC) and making liver resection questionable is unfrequent. The aim of this work is to report on postchemotherapeutic surgical management of 3 cases of HB with PC.

In case 1 the tumor involved the right lobe and segment I, portal division and bile ducts union. Liver blood supply came from a median and a right artery and from 2 cavernomas, each of them following an arterial axis. Surgery consisted of a right lobectomy extended to caudate lobe, with resection of hilum. Vascular supply was ensured by the median artery and its associated cavernoma. Left bile duct was diverted through a Roux-en-Y loop. With a 55 months follow-up, the child is alive in complete remission, without evidence of portal hypertension (PH).

In case 2 the tumor involved the right liver and the posterior part of segment IV, and the portal vein division. Blood supply came from a single artery and from a venous cavernoma. Surgery consisted of a right hemihepatectomy extended to posterior part of segment IV, with removal of the neoplastic portal thrombus and excision of a solitary small nodule of left lobe. With a 15 months follow-up, the child is alive, with a normal AFP value and without evidence of PH.

In case 3 the disseminated tumor was predominantly located in right liver. The first surgical step consisted of a right hemihepatectomy with intraparenchymatous ligation of portal pedicles. After post-operative chemotherapy AFP level decreased and remained in normal values range until the 6th month but increased slowly thereafter. An orthotopic liver transplantation could be achieved 18 months after resection, using aorto-hepatic conduit and mesenterico-portal anastomosis. AFP decreased once more to normal values, but is slowly increasing, without evidence of tumor at successive work-ups.

O-38

IS SURGICAL TREATMENT IN LIPOBLASTOMA ALWAYS REQUIRED?

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Lipoblastoma is an uncommon, benign mesenchymal tumor with an excellent prognosis despite its potential to local invasion and rapid growth. The choice treatment is a conservative and complete excision. The recurrence rate reported is 14%. **Two cases** of lipoblastoma are reported. **B.C.**, a 2½ year-old girl presented an abdominal mass discovered casually. Ultrasound and barium studies confirmed the presence of a mass with a displacement of the ileum into the upper abdomen and an empty left lower quadrant. CT scan showed a large retroperitoneal fatty tumor. Laboratory investigations were normal. At laparotomy an enormous mass (800 gr), strictly enveloping the left hypogastric, the external and common iliac veins was found. A complete tumoral excision caused many injuries to the venous iliac axis and a reconstruction by an end-to-end anastomosis proved necessary. Pathological findings confirmed the intraoperative diagnosis of lipoblastoma. No signs of recurrence were evident at 8 years follow-up. **P.J.**, a 2 day-old boy presented a congenital left upper thigh mass and a limited movement of the hip. Ultrasound and Magnetic Resonance (MR) showed an unencapsulated, undefined fatty lesion occupying the left acetabulum, infiltrating the adductors muscles. A surgical biopsy was performed at the age of 1 month and diagnosis was lipoblastoma. To avoid the risk of mutilating surgery and considering the patient's age we decided to follow the lesion by imaging. A complete spontaneous resolution of lipoblastoma was shown by MR at 1 year follow-up.

Comment: no spontaneous resolutions of lipoblastomas have ever been reported in current literature. The need for a complete surgical excision has never been questioned. In our first case an important vascular damage was necessary to obtain a radical excision; in the second case in order to avoid an hip functional lesion no surgery was performed. These data could suggest that the best therapeutic approach in lipoblastoma should properly take into consideration the patient's age and a feasible conservative surgery.

O-39

INTERLEUKIN-15 RECEPTOR-α IS EXPRESSED IN FRESH CHILDHOOD BURKITT`S LYMPHOMA CELLS

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Interleukin-15 is a newly discovered T-cell growth factor which resembles interleukin-2 in its tertiary structure and in many of its biological functions. To examine whether *IL-15* is involved in the pathogenesis of pediatric Burkitt's lymphoma, we investigated the expression of the *IL-15* cytokine itself, the three chains of the IL-15 receptor, and the proliferative response to IL-15 in a panel of 14 Burkitt's lymphoma cell lines. Expression of IL-15 and its receptor was also studied in 7 fresh highly purified BL-cell preparations. IL-15 cytokine expression and expression of the IL-2/IL-15-β and common γ-chain of the receptor was studied by reverse-transcriptase PCR, when necessary followed by Southern hybridization with a labeled internal oligonucleotide. Proliferation of cells in the presence of IL-15 was studied by nonradioactive proliferation assays. *IL-15* was highly expressed in 3 of 14 cell lines, to a lower extent in 3 further cell lines, and in none of the fresh BL-cell preparations. IL-2 expression was tested as a control and was found to be negative in all cell lines. The IL-2/IL-15 receptor common γ-chain was detected in all Burkitt's lymphoma cell lines. The β-chain showed varying expression by PCR in 6 out of 14 cell lines and in 1 of 7 fresh BL preparations. 3 of 7 highly purified fresh BL-cells expressed the IL-15R α-chain. The IL-2 receptor α-chain was used as a control and was found to be expressed in all except one of the cell lines. IL-15 inhibited growth in 2 of 13 cell lines, but all other cells were unaffected. We conclude, that IL-15 and the three chains of its receptor can be expressed in fresh childhood Burkitt's lymphoma cells and in lymphoma cell lines. IL-15 may be an autocrine growth-inhibitory factor for some Burkitt`s lymphoma cell lines.

O-40

EX VIVO MOLECULAR PURGING BY ANTISENSE OLIGONUCLEOTIDES (AS-ODN) OF BURKITT'S LYMPHOMA SEEDED PERIPHERAL BLOOD STEM CELLS (PBSC)

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Recently, we have demonstrated that 5' AS-ODN (5´-GCAGCACAGCTCGGGGGT3´) combined with lipofectamine (LFM) significantly inhibited the proliferation of Daudi cells (Williams & Cairo et al *Leukemia* 10:1980, 1996). In this study, to develop an alternate method of purging without BM toxicity, tumor seeding (1%, 0.1%, 0.01% of Daudi in PBSC) and elimination of tumor by AS/LFM were assessed by clonogenics and flow cytometry ($CD77^+/CD22^+$). There was a significant decrease in Daudi CFU formation in Daudi/PBSC mixture (1%, 0.1% and 0.01%) (132.7±9 vs 30±2.5 vs 2.8±0.2 colonies/50,000 cells, p=<0.01). By flow cytometry, a 12.2±4% difference of $CD77^+/CD22^+$ cells was seen in 1 log decrements of tumor contamination. AS/LFM treatment significantly inhibited Daudi CFU formation in Daudi/PBSC, but LFM alone and ODN controls (sense: 5´-ACCCCCGAGCTGTGCTGC-3´, and reverse antisense [RAS]: 5´-TGGGGGCTCGACACGACG-3´) did not. AS-ODN/LFM treatment achieved 64.2 ±1.15% tumor depletion.

	Control	LFM	AS-ODN/LFM	Sense/LFM	RAS/LFM
1%	143.8±8.9	109.5±13.7	54.5±5.7*	136±42	145±59
0.1%	27.2±2.5	22.1±4.2	9.2±1*	20±4	20±17
0.01%	2.1±0.3	1.9±0.5	0.75±0.08*	0.8±0.1	1.7±0.2

* p<0.05

By these methods we can detect minimal residual disease in PBSC to 0.01%

tumor contamination (1/10,000). Additionally, there was no inhibition of PBSC committed progenitor proliferation (CFU-GM) by AS-ODN. *Ex vivo* molecular purging with AS-ODN/LFM achieved a greater than one-half log tumor depletion without hematopoietic toxicity. This approach, utilizing specific *ex vivo* molecular purging, could be combined with chemotherapy purging in patients with involvement with Burkitt's lymphoma in BM or PBSC who require autologous stem cell transplantation.

O-41

EXPRESSION OF B7-2 (CD86) MOLECULES BY REED-STERNBERG CELLS OF HODGKIN'S DISEASE

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Ligation of CD28 on T cells with its natural ligands B7-1 (CD80) or B7-2 (CD86) provides a major costimulatory signal for T cells and is of potential importance for tumor rejection. We previously reported a strong expression of B7-1 on Reed-Sternberg cells and anaplastic large cell lymphoma cells. We here report our findings on B7-2 expression by malignant lymphomas (n = 70). B7-2 was present on the neoplastic cells of anaplastic large cell lymphoma in two of three cases studied, and on a subpopulation of the malignant cells in one out of 4 cases of follicular lymphoma. B7-2 was not expressed by the neoplastic cells of the other non-Hodgkin's lymphomas (n = 32), including T cell-rich B cell lymphoma. In contrast, Reed-Sternberg cells in lymph nodes affected by Hodgkin's disease are strongly positive for B7-2 (n = 31). Evidence for a functional correlate of this expression was obtained by our findings that the combination of anti-B7-1 and anti-B7-2 monoclonal antibodies was more effective than each separately in blocking allogeneic T cell activation (proliferation and cytokine secretion) by Hodgkin's disease-derived cell lines as stimulators. The possible role of B7-1 and B7-2 expression for the course and symptomatology of Hodgkin's disease is discussed.

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VARIABLE PROPORTION OF TUMOR CELLS REFRACTORY TO P53 MEDIATED APOPTOSIS: A MECHANISM OF THERAPY RESISTANCE IN EWING TUMORS?

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Tumor cells escaping cytotoxic treatment constitute a potential source of relapse. Several mechanisms contribute to therapy resistance. In healthy tissues, the tumor suppressor gene product p53 is involved in the elimination of cells with damaged DNA generated by genotoxic agents. This pathway of protecting genomic integrity is frequently impaired in many tumors due to mutational inactivation of p53. By contrast, most pediatric neoplasms including the Ewing family of tumors retain biologically active wildtype p53. We applied a transient transfection assay to test apoptotic responsiveness of Ewing tumor cell lines to ectopic wildtype p53 overexpression. By flowcytometric analysis of cells double stained for p53 expression and DNA content we demonstrate highly reproducible differences in apoptotic responsiveness of several Ewing tumor cell lines tested irrespective of endogenous p53 gene status. Tumor cells surviving p53 overexpression for up to 48 hours showed a cell cycle distribution similar to that of untransfected cells with only a small increase in the G1 fraction. Sensitivity to p53 mediated apoptosis did not correlate with cellular growth rate or expression levels of p53, or of members of the bcl2 family antagonistically involved in the regulation of cell death. In stable transfection experiments with conditional p53 constructs, surviving cells could be rescued after a 48 hour p53 induction even from cell lines with the highest apoptotic sensitivity. After expansion of these cells under conditions restrictive for wildtype p53 overexpression the majority of cells regained p53 sensitivity and died in response to p53 induction. Our data indicate that accumulation of wildtype p53 for a limited period of time, as it occurs during radio- and chemotherapy cycles, does not suffice to readily eliminate Ewing tumor cells.

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SELECTIVE EXPRESSION AND CYTOKINE REGULATION OF IMMUNE ACCESSORY ANTIGENS ON EWING´S TUMOUR CELLS: CD40 AND CD80

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CD40 and CD80 are immune accessory antigens involved in T cell activation through interaction with the counterligands CD40L and CTLA4/CD28 respectively. CD40 is expressed on B cells, endothelial cells and carcinomas, while CD80 is selectively expressed on antigen-presenting cells (APCs). Herein we demonstrate the biphasic expression and regulation of CD40 and CD80 on distinct populations of Ewing´s tumour (ET) cells. Flowcytometric analysis of various ET cell lines in vitro revealed a dichotomous "two-cell" pattern with one population of larger (L) cells, together with a second population of smaller (S) cells. The percentage of S-cells in 15 different ET cell lines ranged from 5-50% of the total cell population. CD40 antigen showed prominent expression on most L-cells and moderate to low expression on S-cells. By contrast, CD80 was highly expressed on the majority of S-cells and was minimally expressed on L-cells. Treatment of cells with interferon (IFN)γ and tumour necrosis factor (TNF)α enhanced the expression of CD80 on S-cells but not on L-cells. The cytokines also stimulated the expression of CD40 though mainly in L-cells. CD86, which is another immune accessory antigen, was neither expressed nor upregulated in ET cells. MHC-class I and the immune accessory antigen CD54 (ICAM-1) were equally displayed and upregulated in L- and S-cells. Interestingly, the CD95 (Fas/APO-1) antigen, which upon activation with anti-CD95 mAb CH11 evoked an apoptotic signal in ET cells, was primarily expressed on S-cells and upregulated in both S- and L-cells. In summary, the results indicate that conspicuous features of APCs can be recognised in distinct ET cell populations with S-cells being the more differentiated phenotype of L-cells. The differentiation-inducing cytokines IFNγ and TNFα may play a pivotal role in eliciting a cytotoxic T cell-dependent immune response against ET by enhancing expression of the important costimulatory antigens, CD40 and CD80.

O-44

EVALUATION OF MYOD1 TRANSCRIPT BY RT-PCR IN TUMOR CELL LINES AND RHABDOMYOSARCOMA TUMOR SAMPLES.

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MyoD1, a transcription factor expressed during skeletal muscle

development, has been detected by immunohistochemical analysis and northern blot analysis in normal skeletal muscle and in Rhabdomyosarcoma (RMS).
We developed an assay to evaluate the expression of MyoD1 by RT-PCR with specific primers designed from the human MyoD1 cDNA sequence, which would give a predicted product size of 443 bp. To test the specificity of the assay we analyzed 10 RMS, 3 Neuroblastoma, 2 PNET, 2 Medulloblastoma, 2 B-cell Leukemia and 2 T-cell Leukemia cell lines and normal skeletal muscle. Furthermore, we studied 35 RMS, 1 Leiomyosarcoma, 1 short-term fibroblast culture, 3 peripheral blood stem cell (PBSC) samples and 4 bone marrow samples. MyoD1 RNA was detected in normal skeletal muscle, 8 out of 10 RMS cell lines and 34 out of 35 RMS samples, whereas no expression was found in non-RMS tumor cell lines, Leiomyosarcoma, fibroblast culture, PBSC and bone marrow samples. This study demonstrates that MyoD1 is expressed in nearly all RMS, but not in other tumor cells or tumor samples and that it could represent a specific marker for the evaluation of bone marrow involvement in RMS. This RT-PCR assay could be a useful molecular tool in the diagnosis, work-up and detection of minimal disease in children with small round cell. *Emanuela Frascella is recipient of a fellowship from AIRC.*

O-45

OCCURRENCE AND FUNCTIONAL PROPERTIES OF HEPATOCYTE GROWTH FACTOR IN HEPATOBLASTOMA

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Rapid growth of recurrent disseminated tumor and/or metastases can develop in children after resection of non-pretreated hepatoblastoma (HB) during the period of maximal liver regeneration. We therefore investigated the occurrence and functional properties of the hepatocyte growth factor (HGF) in HB patients.

With ELISA we detected elevated serum levels (>1.0 ng/ml) of HGF in 10/22 HB patients in comparison to healthy children. Supernatants of HB cell cultures ($n=7$) and sera of immunodeficient mice with HB xenografts ($n=5$) did not contain HGF. A significant increase of HGF was measured in sera of 14 children 24-48 hours after liver resection. With immuno-enzymatic staining of 20 HB we could localize HGF to stromal fibroblasts. In contrast, its receptor (C-*Met*) was expressed on epithelial tumor cells. In co-cultures, HB cells stimulated human fibroblasts to an enhanced secretion of HGF. Addition of recombinant human HGF in concentrations of 0.2-15.0 ng/ml to cultures of the 3 HB cell lines HUH 6, Hep T1 and Hep T3 induced a 30% increase of the proliferation activity, whereas concentrations above 50 ng/ml resulted in depression of the cells' growth.

These results indicate that post-operatively secreted HGF can stimulate growth of non-pretreated HB. Furthermore, HB is able to produce HGF as its own growth factor in a paracrine fashion. In high concentration, HGF may become an inhibitor of tumor cell proliferation. These observations could be a basis of new treatment strategies against HB.

O-46

Inhibition of bone marrow metastasis by calmodulin antagonists in mice with C-1300 neuroblastoma

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Calmodulin(CaM) is a ubiquitous, calcium-binding protein that is responsible for many of the intracellular actions of calcium. In this study, the antimetastatic effect of CaM antagonists, such as Trifluoperazine (TFP), Chlorpromazine (CPZ), Tamoxifen (TAM), and Melittin(MEL), was examined using a new experimental model of liver and bone marrow metastases induced by C-1300 neuroblastoma (C-1300 NB) in A/J mice. By ELISA, CaM content of bone marrow matastasis was 7.0 μ g/10^7 cells which was higher than normal bone marrow (4.3 μ g/ 10^7 cells). CaM content of liver metastasis was below a detectable level by this method, while that of subcutaneous leg tumor was 0.67 μ g/10^7 cells. Liver metastasis, where CaM content was low, was not affected by CaM antagonists. A sensitive in vivo tumor intake assay was used to detect bone marrow metastasis, as pathological detection is not reliable. Femoral bone marrow cells including metastasis were harvested ten days after tumor cell injection. To investigate the effect of anti-CaM drugs, the number of tumor spots growing as new subcutaneous tumors on murine abdominal wall, derived by the inoculation of bone marrow content of C-1300 inoculated mice which were treated with a CaM antagonist, was counted. Injection of CaM antagonists 14 days after tumor cell inoculation, inhibited the bone marrow metastasis, where CaM content was high. No tumor growth was identified in mice treated with TAM or MEL. With TFP or CPZ treatment, the occurrence ratio of tumor spots growing after injection of bone marrow content were 10% and 16.7% respectively of that of untreated animals.

These findings raise the possibility that CaM antagonists may have clinical value for the treatment of bone marrow metastasis of NB.

O-47

PRIMITIVE NEUROECTODERMAL TUMOR IN TRANSGENIC MICE CARRYING THE E1 GENE OF ADENOVIRUS WITH THE HUMAN RENIN PROMOTER

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Frequent tumorigenesis was observed in transgenic mice carrying combined E1A and E1B genes of the adenovirus type 12 fused with the deregulated human renin promoter. All the tumors observed were primitive neuroectodermal tumor(PNET) of the identical histology. We investigated the detailed characteristics of the tumors and oncogene expresison. Fused gene of the human renin promoter gene and the combined E1A–E1B gene was introduced into the fertilized eggs of C57BL/6J mice for generating transgenic mice. One hundred and fifty–five transgenic mice out of 400 offspring were obtained by mating the transgenic mice with inbred normal mice. To date tumors were observed in 10 male and 22 female transgenic mice and confirmed histologically as PNET. Among the 155 transgenic mice, abdominal distension caused by marked intestinal dilatation, mimicking Hirschsprung disease, was observed in 10 mice. The sites of origin of the tumors were head, extremities, mediastinum, retroperitoneum, intraperitoneal, pelvic and there were no obvious correlation of the sites of origin to the sympathetic nervous system. All the tumors showed almost identical histology and had characters of primitive neural tissues. Electron microscopy revealed neurosecretory granules and neurite processes. Immunohistochemical study disclosed positive to PGP9.5 and Leu7 but negative

to NSE, NF, tyrosine hydroxylase, GFAP, MIC2, vimentin, desmin and myoglobin, indicating very primitive neuroectodermal tumor. We have investigated which genes or gene families were activated or suppressed in expression in the transgenic mice. The c–myc, N–myc, and L–myc were not amplified in Southern blot analysis but, notably, the 3 sets of myc genes and N–CAM genes coexpressed in the transgenic tumors by Northern analysis. This transgenic mice model will hopefully elucidate the genetic mechanism of tumorigenesis of PNET.

O-48

PRE-SURGICAL RADIOTHERAPY IN EWING TUMORS. MATCHED PAIR ANALYSIS OF 64 PATIENTS FROM THE (EI)CESS STUDIES

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Background: Possible systemic dissemination of malignant disease during surgery is the rationale for neoadjuvant cytostatic drug therapy in Ewing tumors. We evaluated, whether or not additional introduction of pre-surgical radiotherapy was feasible, and could further improve event-free survival.

Patients and methods: Of 661 patients treated for localized Ewing tumor according to the European CESS91P/EICESS92 protocols between 1991 and 1996 by neoadjuvant cytostatic drug therapy and individualized local therapy, 101 received pre-surgical radiotherapy of 48 to 54 Gy paralleling chemotherapy, and followed by surgery 4-8 weeks later. Indications for pre-surgical radiotherapy were poor MRI-detectable tumor response to chemotherapy, and tumors where less than wide surgical margins were expected. 64 patients were evaluable for matched-pair analysis. Control patients matched with respect to tumor site and size, and time under study, were treated according to the same protocols, but underwent either surgery, or radiotherapy, or surgery followed by radiotherapy for local treatment. Complication rates were recorded, event-free survival (EFS) was analyzed by univariate and multivariate procedures.

Results: Pre-surgical radiotherapy is safe with no increase in surgical complications. EFS 5 years after diagnosis with or without pre-surgical radiotherapy was 0.55 and 0.51, respectively. Minor advantages, though not significant, could be found in pelvic tumors, and in small tumors poorly responding to chemotherapy.

Conclusion: Pre-surgical radiotherapy is safe and feasible, but does not seem to significantly improve outcome. Possible indications could be pelvic primaries poorly accessible to surgery, and poor chemotherapy response.

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EXOGENOUS INSULIN-LIKE GROWTH FACTOR-I (IGF-I) DOES NOT EFFECT RHABDOMYOSARCOMA GROWTH NOR THE ANTITUMOR EFFECT OF VINCRISTINE, CISPLATIN, OR DOXORUBICINE.

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We studied the influence of IGF-I on rhabdomyosarcoma growth and on the antitumor effect of cytostatic drugs. IGF-I is a potential protective agent against vincristine neuropathy. However, a protective agent might compromise the antitumor effect of chemotherapeutic drugs. Moreover, activation of the IGF-I receptor by endogenous IGF-I stimulates tumor growth and inhibits apoptosis.
The alveolar and embryonal rhabdomyosarcoma cell-lines Rh30 and Rh1 were cultured in a growth assay, under serum free and serum containing conditions, in the absence and presence of IGF-I, with and without exposure to vincristine, cisplatin, and doxorubicine. After exposure cells were lysed and nuclei were counted.
Our results showed that IGF-I: 1) stimulated Rh30 and Rh1 growth when cultured under serum free conditions; 2) had no influence on Rh30 growth when cultured under serum containing conditions; 3) had no influence on the antitumor effect of vincristine, cisplatin and doxorubicine in Rh30 nor Rh1 cells, when cultured under serum free conditions and data were normalized for differences in cell growth; 4) had no influence on the antitumor effect of vincristine in Rh30 cells when cultured under serum containing conditions.
These results suggest that use of exogenous IGF-I to ameliorate vincristine neuropathy does not adversely effect rhabdomyosarcoma growth nor the antitumor effect of the tested drugs.

O-50

ANTISENSE TO INSULIN-LIKE GROWTH FACTOR RECEPTOR CAUSES REGRESSION OF INTRACRANIAL GLIOMA.

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Insulin-like growth factors and cognate receptor (IGF-1R) play critical roles in differentiation and growth of the developing brain and maintenance of the malignant phenotype in brain tumors. To determine if antisense to IGF-1R antisense inhibits intracranial (IC) tumor growth, we transfected C6 glioma cells with IGF-1R antisense or sense RNA. We evaluated survival for BD-9 rats bearing wildtype C6 IC tumors and compared the effect of IC or flank injections of sense or antisense transfected C6 glioma on wildtype tumor growth. All control rats stereotaxically injected into the caudate nucleus with wild-type C6 died within 28 days. Subcutaneous injection of wildtype or sense-transfected C6 seven days before IC injection of wildtype C6 did not increase survival (IMS 11%, p=0.23). Intracranial injection of antisense C6 did not cause death within 60 days and 2/15 rats had only histologically apparent tumor at 80 days. To determine if IGF-1R antisense reverses the growth of distant wild-type C6 glioma, we injected BD-9 rats with wild-type C6 in right caudate and antisense C6 in left caudate. Concurrent IC injections prevented the growth of wildtype C6 in 11/15 rats (IMS >300%) and initial IC injection of C6 antisense followed 7 days later by wild-type C6 prevented the growth of wild-type tumors in all rats (100% survival at 60 days). Concurrent antisense flank and IC wildtype tumor injections prevented growth of wild-type C6 in 9/15 rats (IMS >300%) and initial flank injection of C6 antisense followed 7 days later by wild-type IC C6 prevented the growth of wild-type tumors in 12/15 rats. The mechanism of IGF-1R -mediated tumor regressions is not well understood; however, pretreatment of rats with cyclosporin or use of athymic (nu/nu) rats reduced but did not eliminate the effects of IGF-1R antisense treatment. Our results show that IGF-1R-mediated signals causing tumor regression are effective regardless of whether the antisense generator is within or beyond the blood-brain barrier. By contrast, impairment of immune function reduces the effectiveness of IGF-1R antisense-mediated tumor regression in C6 glioma.

O-51

^{99m}TC-MIBI IMAGING IN NEUROBLASTOMA: A NEW FUNCTIONAL APPROACH TO P-GP MEDIATED MDR .

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Background: non-localized neuroblastoma (NB) stage 3 and 4, remains a highly chemoresistant disease. Data on expression of P-Glycoprotein (P-gp), causing Multidrug Resistance (MDR) in NB, as determined by current detection methods, remain controversial. In 1993, Piwnica-Worms described the use of Hexakis(2-methoxyisobutylisonitrile)technetium (^{99m}Tc-MIBI) as P-gp substrate.
Objective: The development of an easy and reliable method for the functional testing of P-gp in vitro and in vivo.
Method: Three NB cell lines were tested by immunocytochemistry and flow cytometry using two MoAbs C219 and 4E3. P-gp was demonstrated in >70% of the SK-N-FI and SK-N-SH cells. The third cell line, GI-ME-N is P-gp negative and was used as negative control. ^{99m}Tc-MIBI uptake and efflux, flowcytometric functional tests with rhodamine 123, and modulation with verapamil were compared with the P-gp status of the cell lines.
Results: The in vitro accumulation and retention of ^{99m}Tc-MIBI was significantly higher in GI-ME-N compared to SK-N-FI and SK-N-SH.
In presence of verapamil however, ^{99m}Tc-MIBI accumulation and retention exclusively enhanced in the P-gp positive cell lines. The scintigrafic assays showed an excellent correlation with the flow cytometric functional tests.
Conclusions: Functional studies using ^{99m}Tc-MIBI in absence and presence of verapamil are a reliable alternative to determine the P-gp status of neuroblasts in vitro. Imaging with ^{99m}Tc-MIBI may provide a non-invasive technique to characterize P-gp expression in NB in vivo and perhaps to study P-gp inhibitors. After confirmation in larger studies, this technique may be promising in detecting P-gp in various malignant cells, both in vitro and in vivo.

O-52

KETAMINE ANESTHESIA WITH DIAZEPAM OR PLACEBO PREMEDICATION FOR BONE MARROW PUNCTURES IN CHILDREN WITH LEUKEMIA

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Objective. Ketamine is a potent analgesic drug used for prevention of procedure related pain. Its side effects include emergence phenomena like abrupt falling asleep, unpleasant dreamings, and disturbed vision and hearing at wake-up. We investigated whether diazepam premedication would have a beneficial effect on these side effects.
Methods. A double-blind placebo-controlled randomized cross-over study was conducted in 16 patients with acute lymphoblastic leukemia (11 boys and 5 girls), with a median age of 8.5 years (range 4-16). One hour after oral diazepam (≤15 kg: 2.5 mg, 15-30 kg: 5 mg, >30 kg: 7.5 mg) or placebo, ketamine (1.0-1.5 mg/kg i.v.) was administered immediately before the bone marrow punctures. The patients were monitored a.o. with a pulse-oximeter and observed for signs of anxiety, pain or side effects; also, they were interviewed and asked for their painscore (Oucher) and procedure preference (questionnaire).
Results. The ketamine administration was as safe (monitoring) and effective (painscores) after diazepam premedication as after placebo premedication. No significant differences between the two procedures were noted during the observation of the child. The most observed side effects were irritability (18/32 procedures), diplopia (31/32) and a dry mouth (24/32). Drowsiness and a dry mouth were reported more after diazepam than after placebo premedication in 4 and 5 children respectively. During the actual bone marrow puncture in 7/32 procedures motor action was seen and in 23/32 procedures the children sighed, groaned, cried or talked. In the interview after the procedures 9/16 children considered the procedure with diazepam different: 5/9 less awful dreaming and 4/9 more gradual falling asleep or wake-up. At the questionnaire one week after the second procedure 7/16 mentioned a preference for diazepam, 3/16 for placebo and 6/16 had no preference. The reasons for the diazepam preference were: quick recovery (1), less awful dreaming (2), less anxiety (3), better sleep (1).
Conclusion. Diazepam premedication is safe and ameliorates the side effects of ketamine in half of the patients because of less awful dreaming and more gradual falling asleep and wake-up.

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CHILDHOOD CANCER PAIN: CLINICAL AND BIOLOGICAL ASPECTS

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Ideally, pain should be objectively measured and assessed so that an appropriate intervention can be chosen, and palliative documented without the need for a large amount of clinical experience. Measurements of pain, while important clinically, is imperative in advancing the field of paediatric pain control. Documentation of the efficacy of analgesic techniques is impossible without standardised measures of proven reliability and validity.
The release of beta-endorphin and corticothropic from pituitary gland is stimulated by stress. It is generally accepted that plasma beta-endorphin concentration is a good indicator of stress, but it is not exactly underlined which kind of stress has to be considered.
Opioid receptor types are found in different proportions in different nervous system regions, with good correlation between their anatomical distribution, anti-nociceptive effects, and other side effect. Serum endorphin evaluations seem strictly related to pain intensity, particularly in chronic pain. However, any serial measure in childhood has been carried out, yet.
Aim of the study is to screen the normal serum endorphin ranges in children and its variation with ages, sex, psychic stress, and pain (acute and chronic).
Whole blood samples were collected from pts into vials and plasma immediately removed and stored at -20°C until assayed. Plasma concentrations were measured in double by radio-immuno-assay by use of a anti-human beta-endorphin serum (IRMA - Technical Laboratories Instrumental). A standard curve was drawn and values obtained were compared between different subgroups of patients.
Sixty-four children entered, aged from 9 months to 18 years (33 male, 31 female), fifteen were tested during anaesthesia before surgery, seven were affected with kidney disease, and fourthy-one with tumour. They all were painless, and had previously received a venous prick.
The total mean value was 34.4 pg/ml (range 1-53.3 pg/ml), the mean value in females was 23.8 pg/ml (range: 1-53.3pg/ml), and in males 22.3 pg/ml (range: 1-46.1pg/ml). The mean in all non-oncology pts was 32 pg/ml (range 1-53.3 pg/ml), in the surgical-anesthetised subgroup was 16.8 pg/ml (1-30.6 pg/ml), in nephropatic subgroup was 43.1 pg/ml (36.2-53.3 pg/ml). The mean obtained in the subgroup of patients crying during pricking was 41.6 pg/ml. The mean level in infancy and childhood was 22.5 pg/ml (range 1-48.3 pg/ml) this remained below adult levels until prepuberty, over12 years old, when it reached 36.1 pg/ml (range 18.8-53.3 pg/ml).
Six oncology pts received more than one evaluation on different day sample in the same setting. The mean on lowest values was 12.9 pg/ml (range 1-17.2 pg/ml), and the one on highest values was 20.87 pg/ml (range 4.4-32.7 pg/ml). All these ranged below +2SD of endorphin basal concentration curve.
The values obtained in pain conditions overrode this range, a mean obtained by samples from a group of painful patient was 63.1 pg/ml (range 50.8-95.7 pg/ml). Serum endorphin value in a patient affected with a stage 4 neuroblastoma, rose to 95.7 pg/ml during painful episode (karnofsky: 2, colorimetric scale: 2), then fell to 50.8 pg/ml after attenuation and to 32.7 pg/ml after complete relief (karnofsky: 0, colorimetric scale: 0).
The serum endorphins rising, over the normal range, seem to be strictly related with pain condition. Further evaluation need to relate different values with the intensity of pain.

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TOTALLY IMPLANTABLE CENTRAL VENOUS ACCESS DEVICES FOR PAEDIATRIC CHEMOTHERAPY PATIENTS

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Objective: We reviewed the port devices inserted at our centre between August 1989 and August 1996 to assess their safety and reliability as a means of central venous access in children with cancer.
Results: A total of 149 ports were inserted into 135 patients during the seven years.Of the 149 ports, 43% were for Acute Lymphoblastic Leukaemia, 12% for CNS tumours, 11% for Wilm's tumour, 7% neuroblastoma, 7% rhabdomyosarcoma, 7% lymphoma, 5% Ewing's Sarcoma, 4% primitive neuroectodermal tumours, 2% germ cell tumours and 6% other diagnoses. Median age at insertion was 4.65 years (range 23 days - 18 years). For solid tumours, ports were inserted at diagnosis. In ALL, the port was inserted after induction and prior to the first intensification block. All were placed by open cutdown, 63% in the right internal jugular vein (RIJ), 15% LIJ, right external jugular (REJ) in 15% and LEJ 3%. The port site was usually pectoral (89%) or axilla (10%).Total number of catheter days was 60 785 with median of 357 days (range 3 - 1096). 120 ports were removed, 66% electively as treatment was complete. 8% were infected. Coagulase Negative Staphylococcus was the commonest organism. There was no apparent link between tumour type and line infection. 5% were blocked. 6% were electively changed to a Hickman line due to relapse and plans for future bone marrow transplant.
Conclusions: Ports provide safe, acceptable, longterm access for children on most chemotherapy regimes. Our infection and blockage rates are lower than the 1992 Children's Cancer Study Group audit for lines and ports. They

are comparable with the recent UKCCSG Hickman line audit, however our follow up period is considerably longer. Our experience would concur with the CCSG report which recommended that implantable devices are inserted unless continuous access or bone marrow transplant is expected.

O-55

USE OF URICOZYME® AND OCCURRENCE OF METABOLIC PROBLEMS DURING INITIAL PHASE OF THE LMB 89 PROTOCOL

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Objective : To study the ability of uricozyme® (urate-oxydase) to prevent metabolic problems and / or renal complications, we reviewed data concerning patients (pts) with B cell lymphoma (majority of Burkitt's) treated in France according to the LMB 89 protocol (exclusion of Dutch and Belgian centers where uricozyme® was not used). 519 evaluable pts from 37 centers were registreted between 1989 and 1996. 314 pts had a stage III or IV lymphoma and 96 a B - ALL. LDH level (known in 480 pts) was more than twice the normal level in 238 pts.

In France, usual pratice in the management of the initial phase of treatment associates alkaline hyperhydration, uricozyme® (50-100 u/kg/d) ± furosemide ± albumine. Chemotherapy starts with a prephase called COP (VCR : 1 mg/m^2 d1, cyclosphamide 300 mg/m^2/d1, prednisone 60 mg/m^{2}d1-d7, methotrexate IT d1).

Results : We reviewed clinical files of the patients who had registration of metabolic complications after COP. 34 pts presented either a tumor lysis syndrom and / or a renal dysfunction.

There were 6 girls and 28 boys. 23 pts had a stage III or IV lymphoma and 11 a B-ALL. Before COP, 23 pts had a impairment of renal function and 9 a renal tumoral involvement. 5 pts were oliguric and 5 others had a hydronephrosis. After COP, 21 pts had severe metabolic problems (hyperphosphoremia ≥ 3 mol/l in 16 and / or creatininemia more than three times the normal value in 9).

Renal dialysis was performed in 7 cases (6 lymphomas and 1 B-ALL).

Conclusion : The percentage of dialysis performed (< 2 % pts with st III, IV and ALL) is low, compared to other series of patients with the same disease. The use of uricozyme® in France during the initial phase might explain greatly these results.

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THE PROGNOSTIC SIGNIFICANCE OF INITIAL TUMOUR VOLUME FOR METASTATIC RELAPSE IN PAEDIATRIC SOFT TISSUE SARCOMA

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Purpose: The aim of this retrospective analysis was to investigate the prognostic significance of initial tumour volume in soft tissue sarcoma for incidence of systemic (metastatic) relapse in children and adolescents.

Patients and Methods: Initial CT and MRI films of **54** patients, 34 referred to this abstract, with localised disease and treated within the trials CWS -81, -86 and -91 (n_{total}=651 pts., 32 (5%) with isolated metastatic relapse) were evaluated for tumour volume (calculated by the ellipsoid formula). 3 histologic groups of STS were compared (alveolar [RMA] and embryonal Rhabdomyosarcoma [RME], and peripheral neuroectodermal tumours [PNET]). Due to small population a match pair analysis (1 patient with systemic relapse matched to 1 without by **same characteristics and treatment**) was applied. The patients were selected according the occurrence of metastatic relapse and availability of CT/MRI scans. Metastasis free survival (MFS) analysis was performed in U-Test (Mann-Whitney) as absolute or relative measures (absolute tumour volume [ATV] and relative tumour volume [RTV :referred to body surface area]).

Results: U-Test of MFS revealed a high predictive value of absolute and relative tumour volume for occurrence of metastasis, as shown below.

Histology:	all		RME		RMA		PNET	
metastasis	yes	no	yes	no	yes	no	yes	no
Median vol. cm^3	92,1	32,5	127	31	37,7	15,95	192,5	152,5
n	17	17	7	7	6	6	4	4
p for Δ	**<0,0007**		**<0,02**		**<0,02**		**<0,09**	
cut-off cm^3	-		50		20		180	

Conclusion: Initial tumour volume is an **important** and easily obtainable **prognostic factor** in soft tissue sarcoma - especially for metastatic relapse - and may serve as a basis for risk adapted therapy. It is best represented by the absolute three-dimensional measure in CT and MRI. There seems existence of a cut off point regarding the incidence of metastases in all investigated histologic groups.

O-57

THE COURSE OF THE THYMIC REBOUND PHENOMENON IN BURKITT LYMPHOMA PATIENTS : A RETROSPECTIVE STUDY

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Gallium-67 imaging of Burkitt lymphoma is useful for evaluating the presence of viable tumour in a residual mass after treatment. On the other hand, after cessation of chemotherapy initial thymic rebound has been reported in up to 25% of patients as early as one week, with a range of duration from 2 to 59 months. Hence, we were interested in its temporal evolution to offer the clinician recommendations in the timing of control scans. With this regard, 78 scans of 14 patients (3 girls and 11 boys) aged 2-14 years with Burkitt lymphoma in complete remission were reviewed. Mediastinal (thymic) accumulation was scored subjectively by the same experienced physicians on the scans performed 1 month (t1) , 4-6 months (t4-6), 9-12 months (t9-12) and on further follow-up scans after cessation of therapy. Two patients never showed any accumulation in the mediastinal region before or after treatment. After treatment, 2 other patients showed similar mediastinal accumulation on t1 and subsequent t4-6 67Ga scans and 2 other patients showed a decrease on the t4-6 scans. While the 8 remaining patients showed absent or only slightly increased uptake of 67Ga in the mediastinum on the t1 scan, we observed a rebound phenomenon in the anterior mediastinum on repeated t4-6 67Ga studies. The rating scores between mediastinal 67Ga accumulation respectively on t1 and t4-6 scans were statistically significantly different. Finally 67Ga accumulation in all 12 patients with a positive 67Ga scan, disappeared between 6-12 months and further remained unchanged for at least 1 year. In conclusion, we demonstrated in a homogenous group of patients suffering from Burkitt lymphoma, in 8 out of 14 patients an increasing gallium-67 activity until 4-6 months following slightly abnormal or normal initial scans after cessation of chemotherapy. In all 12 patients with initial rebound, the phenomenon had disappeared after 12 months and scans further remained unchanged.

Clinical acquaintance with this temporal evolution may be important in the timing of control scans.

O-58

THE VALUE OF MAGNETIC RESONANCE IMAGING IN BONE MARROW INVOLVEMENT OF HODGKIN'S DISEASE

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Objective: In this study we compared the effectivity of magnetic resonance imaging (MRI) and bone marrow biopsy in diagnosis of bone marrow involvement in patients with Hodgkin's disease (HD).
Patients and methods: Twenty-one patients with the diagnosis of HD were included in this study. Nine of them were stage III or IV and 12 were previously diagnosed patients with relapse. They were evaluated with bone marrow biopsy and MRI of lumbar vertebrae. The ages of the patients were between 4-24 with a mean of 11. The hemoglobin concentrations, sedimentation rates and lactate dehydrogenase levels were 5-14 (mean 10.5 gr/dl), 15-150 mm/hour (mean 66)and 246-1132 iu/l (mean 647) respectively. The biopsies were taken from anterior superior iliac spine with age-appropriate Jamshidi biopsy needle. At the same time MRI of lumbar vertebrae was done.
Results: MRI revealed decreased signal intensity in T1 weighted images in 7 of 21 patients. On the other hand, bone marrow biopsies showed HD involvement in 3 out of 7. The other 14 patients who had normal bone MRI were negative for HD in their bone marrow biopsies. The patients with positive magnetic resonance imaging and a negative biopsy for HD had bone pains. One of these cases had femoral periosteal reaction in bone survey and the other two had height loss in their lumbar vertebral body.
Conclusion: In HD, MRI can be used to exclude bone marrow involvement. Our study also proved that MRI is a sensitive technique in diagnosing bone marrow involvement of HD. More precise results can be obtained by increasing case number.

O-59

STAGE IV CHILDHOOD HODGKIN'S DISEASE (HD): UPDATED RESULTS OF THE SIOP COLLABORATIVE STUDY.

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In 1988, the SIOP started a study for stage IV HD with several aims: 1. To reproduce at in international level the favourable results of the German studies observed in 31 patients (pts); 2. To confirm that radiation therapy (RT) can be limited to 20 Gray in involved fields after effective chemotherapy; 3. To demonstrate the feasability of such a study at an international level accepting that participating groups may differ in the staging strategy.

The treatment consisted of 2 cycles of OPPA and 4 cycles of COPP followed by RT. Radiation dose to nodes was 20 Gy after good response (GR) (complete or more than 70 % regression of the disease) or 36 Gy after poor response (PR) (< 75 % response), 12-15 Gy to the initially involved liver and 12 Gy to the lungs and kidneys if initially involved with incomplete remission after the 2 OPPA cycles.

Four national groups from Germany, France, Italy and Switzerland participated as well as some hospitals from Austria, Spain and the Netherlands.

Between January 1989 and December 1994, **117 pts entered the study.** Median age at diagnosis was 11, 9 yrs (range 2.5 - 18 yrs). 79 pts (68 %) had B symptoms. The predominant histological subtype was nodular sclerosis. In 71 pts, only 1 extralymphatic organ was involved: lung (55), bone marrow (8), liver (4), kidney (2), bone (2). 40 pts had 2 or 3 involved organs. 31 pts underwent laparotomy, 12 were splenectomized.

At completion of therapy, the pts exhibited GR received 20 Gray to the involved nodes. Only 12 pts received 36 Gy to 40 Gray to one or several nodal areas because of PR. 33 pts received lung RT, 18 pts received liver RT, 3 pts received kidney RT. Only 6 pts showed progression during initial therapy and were given alternative CT.

By January 1997, median follow-up of the pts is 52 months (range 5 - 92). 9 pts relapsed after 9 to 51 months. **The projected 5 year EFS is 86 % ± 4 % and overall survival is 90 % ± 4 %.**

In conclusion: these results show that OPPA-COPP chemotherapy followed by 20 Gray represents a valid therapeutic approach for stage IV Hodgkin's disease.

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O-60

PROGNOSTIC SIGNIFICANCE OF A CONTIGUOUS INVOLVEMENT (CI) IN HODGKIN'S DISEASE (HD) THROUGH MDH 82 AND MDH 90 PROTOCOLS OF THE FRENCH SOCIETY OF PEDIATRIC ONCOLOGY (SFOP)

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Aim of the study : to determine the true prognostic significance of CI in patients (pts) with HD treated with modern therapies in the last fifteen years.
Patients and methods : Between 1982 and 1996, amongst 500 pts treated in MDH 82 and MDH 90 protocols of SFOP, 22 (4,4 %) presented at diagnosis with a CI. There were 9 boys and 13 girls with a median age of 13 years (y) 11 months (mo) (range : 6 y 7 mo to 16 y 3 mo). Lukes Rye histological type was 2 (15 pts), 3 (5 pts), 4 (1 pt), not specified (1 pt). Ann Arbor stage was II (13 pts) and III (9 pts). 17/22 pts had B and/or b symptoms. The type of CI was : lung (10 pts), pericardium and/or pleura (6 pts), chest wall (5 pts) and vertebra (1 pt). The treatments followed the principles of SFOP MDH 82 (9 pts) and MDH 90 (13 pts).
Results : before 1989 : 3 stage II pts and 5 stage III pts received three cycles of MOPP/ABVD and one stage II pt 4 ABVD courses.
After 1989 : 5 stage III pts received 4 VBVP courses ; 4 stage II pts and 3 stage III pts received 2 cycles of MOPP/ABVD. One stage III pt was treated by a stage IV protocol.
Tumor response to chemotherapy was CR in 3 pts, PR > 70% in 14 pts, PR < 70% in 5 pts. 20/22 pts received radiotherapy. Site of radiotherapy was : mediastinum in 20 pts at a dose of 20 Gy (13), 30 Gy (1), 40 Gy (5,) not specified (1) ; lung in 3 pts at a dose of 12, 15 and 40 Gy ; chest wall in 3 pts at a dose of 20 Gy (1 pt) and 40 Gy (2 pts). Radiotherapy was discontinued in one patient because of progression.
Tumor response at the end of treatment was CR in 11 pts, PR > 70% in 7 pts, PR < 70% in 2 pts, relapse on therapy in 2 pts. 15 pts are in CR1 with a median follow up of 3 y 10 mo (range : 7 mo to 12 y). 5 pts relapsed : 2 during treatment (1 died and 1 is alive NED at 6 years) and 3 after therapy at 10, 18 and 22 mo (2 died and 1 is alive NED at 4 y). 2 pts progressed after PR and died at 18 and 36 mo.
Conclusion :
1- CI in HD is clearly associated with B and/or b symptoms : 17/22 pts.
2- The ultimate prognosis of chest wall involvement remains very bad : 4/5 pts died.
3- In other cases of CI the prognosis is not different from the general prognosis of HD : 16/17 pts are alive.

O-61

Bulky Disease and Local Control within Combination Treatment of Hodgkin's Disease based on the Experience of the German/Austrian Hodgkin Study (HD - 90)

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Purpose: To evaluate the effect of bulky disease on local control within combination treatment of pediatric Hodgkin's disease (HD) according to the experience of the German / Austrian HD 90 Study.
Patients and Methods: Between 1990 and 1995 563 pts.(315 boys;248 girls; median age12,9years) with HD were treated according to the stage adapted protocol with modified involved field radiotherapy (mIF RT) and chemotherapy. Pts. were treated according to three groups: group I (stage I,IIA) with 2x OPPA(OEPA) and mIF RT 25 Gy; group II (stage IIB,IIIA,IEA,IIEA) with 2x OPPA(OEPA)/2xCOPP and mIF RT 25Gy; group III (stage IIIB, IV, IIEB, IIIE) with 2xOPPA (OEPA)/4xCOPP and mIF RT 20Gy.Pts.with insufficient tumor regression got a local boost of 5 -10 Gy. 495 pts. were eligible for analysis of lymphnode (Ln) size and spread of disease based on clinical and image information (CT, chest X-ray, ultrasound). Bulky disease was defined as lymph nodes > 5cm or a chest tumor > 33% of the thoracic diameter.Tumor relapse was indicated as "infield" or "out of field" or both.
Results: 1062/4950 investigated areas revealed HD with Ln < 5 cm. In 322/1384 involved areas bulky disease was found (52% mediastinal; 39%cervical; 9% axillary, paraaortal, iliacal) with n=92 (28%) in group I, n=95 (29,5%)in group II, n=137(42,5%) in group III. A boost was performed in 84/322 bulky tumors (26%). Relapses were observed in 29 pts.: 17 with bulky, 12 non-bulky HD. The bulky disease group showed altogether 17 pts. with relapses, 4 of them "infield", 4 "outfield", and 8 "infield" and "outfield" (1 unknown). 7/17 pts. with recurrence and bulky disease at

diagnosis recurred in the area of the bulk, 1/7 in the bulk area alone, 6/7 additionally in 1-3 areas. The remaining 9/17 did not recur in the area of the bulk, but in other areas (1-2). In one case there was only local relaps of the bulk, in 6 cases a local bulk relaps plus an infiltration of 1 to 3 areas. In the non-bulky disease group, there were altogehter 12 pts. with relapses, 3 of them "infield"-, 4 " outfield relaps" and 5 " infield and out-field" relapses. 1 to 4 areas were involved..One patient had dissemination of hodgkin's disease.

Conclusion: Bulk seems to have no major effect on local control within systematic combination treatment of pediatric HD according to the experience of (HD 90).

O-62

Randomized Trial of Recombinant metHuG-CSF in an Intensive Program for T-cell Leukemia and Advanced Stage Lymphoblastic Lymphoma. A Pediatric Oncology Group Study (POG #9398).

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We assessed the impact of r-metHuG-CSF on the degree of neutropenia, hospitalizations, and delays in subsequent administration of chemotherapy in 89 patients intensively treated with T-cell advanced stage non-Hodgkin's lymphoma (NHL) or leukemia (ALL) enrolled on POG #9398. This open label prospective randomized pilot trial incorporated G-CSF at 10 ug/kg/day SQ into remission induction phase and two consecutive continuation treatment cycles. Induction included vincristine, prednisone, doxorubicin and cytoxan, followed by a course of high dose cytarabine continuous infusion and L-asparaginase. Continuation cycles included vincristine, prednisone, doxorubicin and 6-mercaptopurine, followed by a course of continuous infusion of high dose cytarabine. Fifty-six patients with ALL and 33 with NHL were randomized either to receive (arm A) or not to receive (arm B) G-CSF. During induction, no statistically significant differences were found in median numbers of days of ANC <500, hospitalization, or of delays in subsequent chemotherapy between arm A (4.5, 9, and 0 days) or arm B (4.5, 9, and 0 days) ($p=0.35$, 0.96, and 0.11, respectively). During continuation cycles, the median number of days of ANC <500 was significantly shorter on arm A (6 days) than on arm B (11 days) ($p=0.017$). However, this did not statistically impact the median days of hospitalization nor delays in therapy ($p=0.22$, and 0.16, respectively). In conclusion, G-CSF may shorten periods of neotropenias during continuation therapy but does not significantly affect duration of hospitalization nor speed the delivery of therapy. G-CSF use should be reserved to treatment programs where significant impact on outcome has been previously documented.

O-63

FATAL LATE EVENTS AFTER TREATMENT FOR PEDIATRIC HODGKIN'S DISEASE (HD).

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We studied fatal late events in 387 patients (pts) treated for HD on 4 consecutive studies at SJCRH (1962-1990). Events consisted of relapsed or refractory HD, second malignancy (SM), or death from any cause. The cohort comprised a total of 5506 person-years. Median age at diagnosis was 14.4 yr (range, 3.0-25.4); 222 were males and 330 were white. Treatment comprised chemotherapy alone ($n=15$), radiation therapy alone ($n=116$), or combined modality therapy ($n=256$). As of 1/31/97, 316 (82%) pts were alive, with a median follow-up of 14.9 yr (2.9-28.3). The 71 fatal events were attributed to HD (31 pts), SM (14), infection (12), accident (7), cardiac disease (6), and complications of asphyxia resulting from laryngeal papilloma (1). The decline in event-free survival for the whole cohort from $81\%\pm2\%$ at 5 yr to $58\%\pm8\%$ at 25 yr was due to factors other than HD recurrence in patients followed for $\geq$ 10 yr. Cumulative incidence rates of cause-specific deaths at 25 yr were $8.6\%\pm1.5\%$ for HD, $8.5\%\pm2.7\%$ for SM, $4.2\%\pm1.8\%$ for cardiac disease, $5.4\%\pm1.7\%$ for infections, and $2.1\%\pm0.8\%$ for accidents. Male pts had a higher ($p=0.02$) cumulative incidence of death from primary disease than did female pts (20-yr estimate:$11.6\%\pm2.3\%$ vs. $4.5\%\pm1.7\%$). In contrast, the cumulative incidence of SM was greater ($p=0.001$) for female than for male pts (20-yr estimates:$20.6\%\pm4.5\%$ vs. $6.2\%\pm2.5\%$). Standardized incidence ratios (with 95% confidence intervals) showed excess risks of 14-fold (9-20) for any SM, 83-fold (17-244) for acute myeloid leukemia, 13-fold (8-19) for solid tumors, and 29-fold (9-67) for breast cancer. Standardized mortality ratios also showed excess mortality from cardiac disease (23-fold;8-50) and infection (33-fold;17-58). Thus, late events contribute to excess mortality and substantially affect the ultimate cure rate for pediatric HD, underscoring the need for further refinements in therapy.

O-64

DO WE NEED GENTAMICIN LEVELS? - AN EFFECTIVE SINGLE DAILY ANTIBIOTIC REGIMEN FOR FEBRILE NEUTROPENIA

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Objectives: A prospective audit has been carried out from July 1996, when the empiric antibiotic policy for children with febrile neutropenia was changed from thrice daily ceftazidime and gentamicin to single daily ceftriaxone and gentamicin. Methods: A serum creatinine and blood cultures were taken at admission. Gentamicin was infused over 30 mts at 7mgs/kg and ceftriaxone at 80mgs/kg (maximum dose = 4g) as a bolus. Gentamicin trough levels were performed by immunoassay 6-14 hours after the start of the first infusion and repeated in those receiving gentamicin for longer than 5 days. Persistence of fever beyond 48 hours was taken as failure of therapy. Serial creatinine levels were performed to detect renal toxicity and pure tone audiometry in those older than 5 years to detect ototoxicity. Results: 80 episodes of febrile neutropenia in 52 patients aged 9 mts to 14 yrs have been treated to date. 49 of the 61 (76%) in whom no organism was isolated, responded to the single daily regimen. Of the 19 (24%) culture positive cases, 10 had organisms sensitive to one or both antibiotics. The serum creatinine levels were normal in all cases. The trough gentamicin level was non-toxic in 79. In one case, a borderline level was obtained from an indwelling port and a subsequent sample was non-toxic. Pharmacokinetic studies (at 1, 3, and 8 hours) done on 14 patients showed a peak of serum gentamicin levels within an hour of infusion. Mean peak levels were 12mg/l and trough less than 2mg/l. No renal toxicity has been observed and pure tone audiograms performed on 20 children are normal. Conclusions: In this study, 74% of the children responded to the once daily regimen and none had recurrence of infection. Failure was seen in the those with staphylococcal (9) or anaerobic (1) infections. The regimen is safe, effective and cost beneficial. Adequate post antibiotic effect was demonstrated in all cases including those who were finally treated with gentamicin monotherapy. Though further data needs to be collected in children, in the presence of normal renal function, the estimation of a trough gentamicin level is probably unnecessary in those receiving single daily gentamicin infusions.

O-65

PREVENTION OF GRAM-POSITIVE INFECTIONS WITH TEICOPLANIN DURING THE INDUCTION THERAPY OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) : RESULTS OF A PROSPECTIVE RANDOMIZED TRIAL.

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Objective : About 60% of the pts with ALL are afebrile at diagnosis. The induction therapy lead to a severe and prolonged neutropenia (< 0,5 x 10^9/L) resulting in documented fever or fever of unknown origin (FUO) in most cases. A combination of antibiotics is usually administrated until neutropenia resolved. Gram-positive infections are the most commonly isolated organisms and fatal streptococcus septicemia are reported. In order to evaluate if the prophylactic use of teicoplanin could decrease the infection morbidity, and limit the use of empiric antibiotherapy in children undergoing an ALL induction therapy, we conducted a randomized trial, in patients without fever at diagnosis.

Patients and Methods : Children coming from 7 french centers were enrolled in this study. Patients who did not develop a neutropenia < 0,5 x 10^9/L within the 15 days following the inclusion were excluded. The ALL treatment consisted in the french FRALLE 93 protocol using predinosolone, vincristine, idarubicin or daunorubicin, and L.asparaginase. After a 24-hour observation period, pts who remained afebrile were randomly assigned to receive teicoplanin (10 mg/kg o.d. IV after initial loading dose of 10 mg/kg every 12 h for 3 doses) until neutropenia resolved, or no prophylaxis. In case of fever, patients received the combination of ceftazidime and netilmicin in both groups, plus teicoplanin in the group without prophylaxis.

Results : Between November 1994 and October 1996, 68 pts aged 2 months to 16 years (median: 5 years) were enrolled ; 18 were excluded because neutropenia did not occur within 15 days ; 50 were eligible, 25 received teicoplanin. Both groups are comparable according to age, pronostic group of ALL, severity and duration of neutropenia (< 0,5 x 10^9/L = 28 days; < 0,1 x 10^9/L = 12 days). 5 FUO and 4 documented infections (2 Gram-positive) were observed in the group with teicoplanin versus respectively 13 and 7 (5 Gram-positive) in the group without prophylaxis ($p < .004$ for fever episodes ; $p < .05$ for FUO ; p NS for Gram-positive infections). The median time before the onset of the first fever was 26 days in the group with teicoplanin versus 13 days ($p < .05$).

Conclusions : Prophylactic teicoplanin significantly delayed the onset of fever, caused a significant decrease in the number of fever episodes, and in the number of days of empiric antibiotherapy. The total cost of antibiotics in each group is under study.

O-66

RANDOMISED COMPARISON OF TWO DIFFERENT TIME-BASED SCHEDULS IN THE TREATMENT OF INFECTIOUS EPISODES IN IMMUNOCOMPROMISED CHILDREN

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Severe infections during bone marrow aplasia after polychemotherapy are common complications in pediatric oncology. We investigated, if shorter intervalls of application of piperacillin (Pip) and netilmicin (Net) on the first day of antimicrobial therapy can reduce the necessity of other antibiotics.

From January 1994 to December 1996 84 children with 149 episodes of fever (>38.5°C) and granulocytopenia (granulocytes < 500/µl) were randomised into two groups of different antibiotic regimens. In both groups we used Pip (85 mg/kg/single dose) and Net (2 mg/kg/single dose). Group 1 (standard group) received Pip at time **0**; **8**; 16; 24 etc and Net at time **4**; **12**; 20; 28 etc. Group 2 (intensified group) received Pip at time **0**; **4**; 12; 20; 28 and Net at time **5**; **8**; 16; 24; 32 etc. After 72 hours we substituted Pip by Imipenem (Imi: 15 mg/kg/single dose, 6-hourly) and after 120 hours we added Amphotericin B (0.5-1.0 mg/kg/day) if fever was not resolved.

Results: There was no difference between the two groups regarding underlying disease, duration and severity of granulocytopenia, number of central venous catheters, positive blood cultures, the reason for fever and side effects. Significant differences are shown in the following table:

	Group 1	Group 2	
Fever episodes (patients)	70	79	
Episodes without antimycotics (patients)	62	67	
Successful treatment without Imi (patients)	49 (79 %)	63 (94 %)	($p < 0.05$)
Successful treatment with Imi (patients)	13 (21 %)	4 (6 %)	($p < 0.05$)
Duration of fever (days)	2.5	1.9	($p < 0.05$)

By shortening the treatment intervalls at the first day of antimicrobial therapy, it was less often necessary to switch from Piperacillin to Imipenem. The patients recovered earlier from fever. The number of fungal infections was equal in both groups.

O-67

TAXOL INDUCES WIDE-SPREAD NECROSIS IN RHABDOMYOSARCOMA

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TAXOL, a microtubule-stabilizing drug which blocks cycling cells in mitosis, exerts selective antitumor activity against ovarian, breast, and non-small cell lung cancers. However, the potential of taxol in the treatment of pediatric cancers remains undefined. The objective of this work was to examine taxol's effect on rhabdomyosarcomas. We utilized four cell lines and two established primary tumors as xenografts transplanted into SCID mice. Taxol alone, at 15 mg/kg x 3 doses, produced massive tumor reduction (10 to 100%), enhanced longevity (minimum one month), and in certain cell types offered cures for animals with either alveolar or embryonal rhabdomyosarcoma (RH-1 50%; RH-30B 10% long-term survival, n≥10). In contradiction to the apoptotic mechanism of cell death described in other tumors after taxol treatment, and confirmed by us with TUNEL method for two breast cell derived xenografts, five out of six cell types demonstrated wide-spread necrosis (>90%), with insignificant apoptosis (<5%) within 48 hours following treatment (n≥10). The sensitivity to taxol varied among cell groups despite low MDR-1 gene expression. One of the embryonal cell lines, RH-1, displayed the most sensitivity with a 50% cell kill at 1.6 nM, which was two log folds higher than that described previously in either breast or ovarian carcinoma cells. At 10 mg/kg x 2 doses, melphalan, a drug being used currently to treat these tumors, caused no tumor reduction in all six cell types and no necrosis within 48 hours except for one alveolar cell lineage (24%). In conclusion, taxol had a defined effect on rhabdomyosarcoma and the treatment appeared to be more efficacious than melphalan.

O-68

PHASE I AND PHARMACOLOGICAL STUDY OF IRINOTECAN (CPT-11) IN CHILDHOOD SOLID TUMORS. G. Vassal[1], F. Doz[2], D. Frappaz[3], K. Imadalou[1], F. Namouni[2], A. Santos[1], D. Mignard[4], F. Pein[1]. [1]IGR, Villejuif ; [2]Institut Curie, Paris ; [3]Léon Bérard, Lyon ; [4]Bellon - Groupe Rhône-Poulenc Rorer, Montrouge, France.

Irinotecan, a specific DNA-topoisomerase 1 inhibitor, is available in adult colorectal cancer in Europe at the dose and schedule of 350 mg/m² every 3 weeks. Preclinical experiments have suggested a